Radiochemical Purity of Technetium-99m Radiopharmaceuticals: Measurement by High Performance Liquid Chromatography

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To

Mary,

Lorna and Andrew

Contents

Declaration

This thesis has been composed by me and the work described in it is my own.

Signed

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Abstract

High performance liquid chromatography (HPLC) has been reported as a means of demonstrating the multicomponent nature of ^{99m}Tc radiopharmaceuticals. HPLC has not, however, been widely reported as a satisfactory technique for measuring the radiochemical purity (RCP) of ^{99m}Tc radiopharmaceuticals. Inadequate techniques based on thin-layer and paper chromatography are in widespread use for this purpose. The aim of the work described in this thesis was therefore to carry out a scientific investigation to establish if quantitative HPLC is a technique that can be used to measure the RCP of ^{99m}Tc radiopharmaceuticals.

An on-line radiation detector was developed and evaluated. This was constructed from a length of column outlet tubing positioned inside the well or across the face of a sodium iodide crystal scintillation detector. The different configurations of tubing and crystal were used to create different sensitivities of detection. Chromatograms were recorded with either a counter-ratemeter/printer system or a computer-based multichannel analyser. At 6,000 counts per second, the highest count-rate encountered in the course of the work, the loss due to dead-time in the multichannel analyser was satisfactorily low at 0.5%.

For successful measurement of RCP, none of the radiolabelled species in a radiopharmaceutical must be retained on the HPLC column and thereby escape detection. A technique for measuring recovery from the column was therefore developed. This involved injecting the sample onto the column with a loop-valve injector, collecting the eluate and measuring the count-rate from it relative to the count-rate from an accurately pipetted sample of the radiopharmaceutical.

An HPLC technique was developed for measuring the RCP of ^{99m}Tc-MAG_3. This used a 250 x 5 mm ODS column eluted with ethanol/10 mM phosphate buffer pH6 (5:95) for 10 minutes then methanol/water (90:10) for 10 minutes. This technique revealed the presence of 6 radiolabelled species in ^{99m}Tc-MAG_3. Recovery from the column was consistently in the order of 100%. HPLC was shown to be superior to both a thin-layer and a paper chromatographic technique and was then used to demonstrate that, contrary to previously published data, ^{99m}Tc-MAG_3 is stable for 6 hours. This finding was confirmed with clinical studies in 40 patients. HPLC was then used to investigate the effect on RCP and stability of various conditions that might arise during routine preparation of ^{99m}Tc-MAG_3.

RCP and stability were not found to be affected by the volume of ^{99m}Tc generator eluate used during preparation, dilution, agitation, the introduction of air or the use of a ^{99m}Tc generator eluate with a ^{99}Tc:^{99m}Tc ratio of 16.

The search for an HPLC technique for measuring the RCP of ^{99m}Tc-DMSA was unsuccessful since this radiopharmaceutical was found to adsorb strongly on three column packings. However, a number of discoveries were made. A previously published HPLC method for analysis of ^{99m}Tc-DMSA was shown to be unsatisfactory. Contrary to previous findings, the level of ^{99}Tc in the ^{99m}Tc used to prepare ^{99m}Tc-DMSA was not found to affect the radiopharmaceutical. ^{99m}Tc(V)-DMSA was shown to be a common impurity in ^{99m}Tc-DMSA with the concentration of this impurity decreasing with time. The method of preparation was shown to influence the ^{99m}Tc(V)-DMSA content of ^{99m}Tc-DMSA.

An HPLC technique was developed for measuring the RCP of ^{99m}Tc-exametazime. This was carried out by gradient elution of a 150 x 4.6 mm PRP-1 column. The gradient consisted of 20 mM phosphate buffer pH 7.4 (100%) to phosphate buffer/tetrahydrofuran (75:25) over six minutes, then held for 4 minutes. This technique revealed five radiolabelled species in ^{99m}Tc-exametazime. Recovery from the column was consistently in the order of 100%. HPLC was found to give comparable results to an established technique in which both thin-layer and paper chromatography are used. HPLC was then used in the development of a routine technique for using sodium iodide to stabilize ^{99m}Tc-pertechnetate that is dispensed for the preparation of ^{99m}Tc-exametazime and to confirm that RCP of ^{99m}Tc-exametazime is influenced by the age of ^{99m}Tc-pertechnetate from which it is prepared but not by the brand of generator from which the ^{99m}Tc is obtained.

The conclusion from this work is that HPLC is a satisfactory technique for measuring the RCP of ^{99m}Tc radiopharmaceuticals.

1. Introduction

1.1 Technetium-99m radiopharmaceuticals

A radiopharmaceutical is a medicinal product which incorporates a radionuclide. Radiopharmaceuticals are widely used in both the diagnosis and treatment of disease and are fundamental to the branch of medicine known as Nuclear Medicine.

The most commonly used radiopharmaceuticals contain the radionuclide technetium-99m (^{99m}Tc). These are employed in the detection of disease by the technique of radionuclide imaging. The simplest radionuclide imaging techniques to investigate particular organs of the body involve administering to the patient a radiopharmaceutical which localizes in the tissue of interest. This can be healthy or diseased tissue depending upon the mode of action of the radiopharmaceutical. Administration is usually, but not exclusively, by the intravenous route. Once the radiopharmaceutical has localized as required, the gamma-rays that are emitted can be detected from outside the patient's body with a gamma-camera. From the spatial distribution of the gamma-rays detected, this instrument constructs an image of the distribution of the radiopharmaceutical in the part of the body that is under investigation. From this image, the presence of disease may be seen. This basic technique is known as static imaging and provides essentially anatomical detail of the organ being investigated e.g. the detection of thyroid nodules, skeletal metastases and pulmonary emboli, although the manner in which the radiopharmaceutical is handled by the organ can provide information on function e.g. the relative uptake of a radiopharmaceutical by each kidney is used as an indication of relative kidney function.

The more sophisticated technique of dynamic imaging involves using a gamma-camera to record the concentration of a radiopharmaceutical in an organ over a period of time to provide functional information e.g. the assessment of kidney function by following the time course of excretion of a radiopharmaceutical that is excreted by glomerular filtration or tubular secretion and the measurement of cardiac function by imaging the blood pool of the heart throughout the cardiac cycle.

Radiopharmaceuticals containing ^{99m}Tc are available for the investigation of most organs of the body e.g. brain, gall bladder, heart, kidneys, liver, lungs, skeleton, spleen, stomach and thyroid.

A typical radiopharmaceutical is composed of two parts - an organ-specific component and a radionuclide. After the radiopharmaceutical has been administered to the patient, the organ-specific component is the means by which the radionuclide is transported to the part of the body that is under investigation. Various organ-specific agents are used in ^{99m}Tc radiopharmaceuticals e.g. particles, colloids, relatively simple molecules, proteins and antibodies. The radiation that is emitted by the radionuclide is the means by which the distribution of the radiopharmaceutical is imaged. A number of factors account for the pre-eminence of ^{99m}Tc as a radionuclide for diagnostic imaging:

- *It has a half-life of 6 hours.* This is short enough to result in only a modest radiation dose to the patient yet long enough to allow most investigations to be performed.

- *Only gamma radiation is emitted.* This avoids subjecting patients to the high radiation doses that are associated with beta or alpha emitters.

- *The gamma radiation has an energy of 140 keV.* This is ideally suited to detection with a gamma-camera.

- *It can be obtained from a radionuclide generator.* This makes ^{99m}Tc relatively cheap in comparison to radionuclides that must be prepared in a cyclotron or nuclear reactor and readily available in the high activities that are needed for imaging procedures.

During the early stages of development, a ^{99m}Tc radiopharmaceutical is often prepared from individual ingredients which are weighed out and processed when required. This is commonly referred to as an "in-house" preparation. However, experience has shown that radiopharmaceuticals only become used widely if they are available commercially. To gain widespread acceptance, the procedure by which a ^{99m}Tc radiopharmaceutical is prepared must be relatively straightforward and sufficiently robust to result in a product of the required quality regardless of the variations in preparative technique that will inevitably exist from radiopharmacy to radiopharmacy. The way in which this has been achieved by the radiopharmaceutical industry is through the development of the radiopharmaceutical kit. This is a vial or combination of vial and ampoules and/or pre-filled syringes containing the ingredients required for the preparation of the radiopharmaceutical with the exception of the

radionuclide. In the case of ^{99m}Tc radiopharmaceuticals, a typical kit is a multi-dose vial that contains a sterile freeze-dried powder.

The first step in the preparation of a ^{99m}Tc radiopharmaceutical is elution of a ^{99m}Tc generator. This provides Sodium Pertechnetate [^{99m}Tc] Injection which is a sterile solution of sodium pertechnetate (^{99m}Tc) in Sodium Chloride Injection. The volume of Sodium Pertechnetate [^{99m}Tc] Injection that contains the required activity of ^{99m}Tc is then injected into the radiopharmaceutical kit. Sodium Chloride Injection can also be added to achieve a suitable radioactive concentration. For most radiopharmaceuticals, labelling of the ^{99m}Tc to the organ-specific component is instantaneous but in a few cases the reconstituted kit must be incubated at room temperature or placed in a boiling water-bath for a specified time to promote the labelling reaction. After preparation, the kit can be issued from the radiopharmacy as a single or multi-dose container depending on the activity of ^{99m}Tc that has been used for reconstitution. Alternatively, aliquots of the contents of the reconstituted kit can be dispensed into syringes or vials to provide individual patient doses which are then despatched to the nuclear medicine department.

The manufacturers of kits subject their products to rigorous quality assurance procedures to demonstrate that radiopharmaceuticals of high quality are produced. However, it is impossible for a manufacturer to anticipate all the possible conditions to which its product might be subjected during use. Several apparently innocuous factors are known to have deleterious effects on the quality of ^{99m}Tc radiopharmaceuticals:

- Haney et al. (1971) and Chia & De Schrijver (1982) have shown that the presence of excessive levels of aluminium in ^{99m}Tc generator eluates that are used in the preparation of ^{99m}Tc sulphur colloid injection can cause instability of this radiopharmaceutical.

- various authors have shown that ^{99m}Tc radiopharmaceuticals can adsorb to the interior surface of particular glass vials (Porter et al., 1975, Millar, 1984, Millar & Stewart, 1985).

- Slater et al. (1983) have demonstrated that when a Gillette syringe is used during the preparation of ^{99m}Tc-tin colloid, a radiolabelled impurity can be formed in the radiopharmaceutical due to substances leaching from the rubber plunger of the syringe.

- Sampson and Keegan (1985) have shown that the radiochemical purity of ^{99m}Tc-pentetate is influenced by the presence of air and the preparation of the radiopharmaceutical with ^{99m}Tc from a generator which requires an eluent that contains an oxidant.

- Hilditch et al. (1986) have shown that agitation during transport can induce an increase in the particle size of ^{99m}Tc-tin colloid.

- Murray et al. (1986) have shown that the use of a bactericidal swab containing chlorhexidine and cetrimide to disinfect the caps of dimercaptosuccinic acid (DMSA) kits can cause the formation of a ^{99m}Tc-labelled impurity in ^{99m}Tc-DMSA.

- Palmer (1992) has shown that the introduction of chlorhexidine into a kit during the preparation of ^{99m}Tc-hydroxymethylene diphosphonate produces an unacceptably high level of ^{99m}Tc-labelled colloidal impurity.

Each of these phenomena is outwith the control of the radiopharmaceutical kit manufacturers and it would be unreasonable to expect a manufacturer to investigate all the combinations of generator, syringes, needles, swabs, vials, Sodium Chloride Injection, etc that might be used in different radiopharmacies during the reconstitution of each of its products. Consequently, the responsibility for ensuring that the procedure and materials used to prepare a radiopharmaceutical result in a satisfactory product lies with the radiopharmacy in which preparation is undertaken. A quality assurance programme to demonstrate satisfactory radiochemical purity is therefore a vital component of the work in any hospital radiopharmacy.

The aspect of quality that gives rise to many of the problems outlined above is the radiochemical purity of the radiopharmaceutical. Radiochemical purity is defined in the British Pharmacopoeia 1993 (B.P.) as:

> *The ratio, expressed as a percentage, of the radioactivity of the radionuclide concerned that is present in the source in the chemical form declared to the total radioactivity of that radionuclide present in the source.*

For the majority of ^{99m}Tc radiopharmaceuticals, the B.P. stipulates that radiochemical purity must be not less than 95%. The consequence of low radiochemical purity is that the ^{99m}Tc does not behave in the required manner after the radiopharmaceutical has been administered to the patient. For example,

- the presence of ^{99m}Tc-pertechnetate impurity in a radiopharmaceutical will give rise to the localisation of ^{99m}Tc in the thyroid, salivary glands, stomach, kidneys and bladder. If a significant amount of this impurity is present in the lung imaging radiopharmaceutical ^{99m}Tc-macroaggregated albumin, the resulting ^{99m}Tc in the stomach can interfere with the detection of lung disease since the stomach lies behind the left lung. The presence of ^{99m}Tc-pertechnetate impurity can give rise to similar problems in cardiac and bone imaging.

- the presence of colloidal ^{99m}Tc in a radiopharmaceutical results in ^{99m}Tc becoming localised in the liver, spleen and bone marrow. If a significant amount of this impurity is present in a bone imaging radiopharmaceutical, those parts of the skeleton in the vicinity of the liver and spleen are not distinguishable. In a similar manner, colloidal ^{99m}Tc in a radiopharmaceutical for kidney imaging can interfere with detection of the right kidney since the liver lies in front of this organ.

- in the cerebral blood flow imaging radiopharmaceutical ^{99m}Tc-exametazime, the higher the concentration of the impurity described as secondary ^{99m}Tc-exametazime complex, the lower the uptake of ^{99m}Tc in the brain.

The techniques that have been developed for measurement of the radiochemical purity of ^{99m}Tc radiopharmaceuticals are almost exclusively chromatographic with thin-layer, paper, gel filtration and electrophoresis being the most common.

1.2 Thin-layer and paper chromatography techniques

Thin-layer and paper chromatography have been widely reported as convenient methods for determining the radiochemical purity of ^{99m}Tc radiopharmaceuticals (Billinghurst 1973,

Pauwels & Feitsma 1977, Zimmer and Pavel 1977, Zimmer et al. 1982, Robbins 1983 & 1984). With few exceptions, these techniques depend upon either the radiopharmaceutical and some impurities being retained at the point of application while other impurities migrate with the solvent front or some impurities being retained at the point of application while the radiopharmaceutical and other impurities migrate with the solvent front. In practice, it is often necessary to use both techniques to identify the common impurities that can be present in a ^{99m}Tc radiopharmaceutical. After development, the relative amounts of the *"chemical form declared"* and impurities are determined from the distribution of radioactivity along the chromatogram. This can be determined either by using a chromatogram scanner and integrating the areas of the peaks or by cutting the chromatogram into transverse strips, measuring the count-rate from each strip in a gamma-counter and thereby quantifying the relative activities in the peaks. This latter technique is commonly referred to as the "cut-and-count technique".

A typical example is the method described in the B.P. for determination of the radiochemical purity of Technetium [^{99m}Tc] Pentetate Injection. In this technique, a first chromatogram is run on Instant Thin Layer Chromatography - Silica Gel (ITLC-SG, Gelman) using butan-2-one as the solvent. In this system, ^{99m}Tc-pentetate and unbound reduced ^{99m}Tc impurity remain at the origin while ^{99m}Tc-pertechnetate impurity migrates with the solvent front. From this chromatogram, the level of ^{99m}Tc-pertechnetate impurity can be determined. A second chromatogram is run on ITLC-SG using 0.9% sodium chloride in water as the solvent. In this system, the unbound reduced ^{99m}Tc impurity is retained at the origin while ^{99m}Tc-pentetate and ^{99m}Tc-pertechnetate impurity migrate with the solvent front. From this chromatogram, the level of unbound reduced ^{99m}Tc impurity in the radiopharmaceutical can be determined. Radiochemical purity is then calculated by subtracting the sum of the levels of ^{99m}Tc-pertechnetate and unbound reduced ^{99m}Tc impurities from 100. Similar techniques are specified in the B.P. for determination of the radiochemical purity of Technetium [^{99m}Tc] Tin Pyrophosphate Injection and Technetium [^{99m}Tc] Medronate Injection.

Thin-layer techniques such as the one described have a number of advantages:

- *migration of the solvent up the ITLC-SG plate is fast.* The analysis is therefore performed quickly. Given that most ^{99m}Tc radiopharmaceuticals are formed through chemical reduction of the Tc, the complexes can be susceptible to oxidation. A rapid technique therefore limits the time for degradation of the radiopharmaceutical during the analytical procedure. A

rapid technique also makes it possible, if necessary, for the radiopharmaceutical to be analysed before it is administered to the patient.

- *the ITLC-SG stationary phase is easily cut.* This makes it particularly suitable for the cut-and-count technique.

- *the ease with which ITLC-SG can be cut allows small plates to be used.* This has been exploited in the miniaturized techniques that have been developed (Zimmer and Pavel 1977, Zimmer et al. 1982). Plates measuring 1 x 6 cm are used commonly in these techniques. In addition to developing rapidly, the small chromatograms make these techniques inexpensive to perform.

The thin-layer techniques are not without their problems. Results have been shown to be influenced by a range of technical artifacts such as size, drying and placement of the sample (Kowalsky & Creekmore 1982) and two multicentre trials have shown wide variations in results obtained (Eckelman et al. 1981, Millar 1986). However, the major disadvantage of these techniques is that they are specific for radiolabelled impurities, not the desired radiolabelled species i.e. the *"chemical form declared"* as it is termed in the definition of radiochemical purity. They would therefore be more properly described as tests for ^{99m}Tc-pertechnetate and unbound reduced ^{99m}Tc impurities than tests for radiochemical purity. It is quite conceivable that an impurity with identical chromatographic behaviour to the active chemical form could be present in the radiopharmaceutical and yet remain undetected. The limitation of these techniques is that species are either retained at the origin or migrate with the solvent front. Reliance on this crude origin/solvent front approach cannot result in a specific technique.

A much more satisfactory situation would be to have a chromatographic system in which each species in the radiopharmaceutical exhibits a specific characteristic i.e. Rf value in the case of thin-layer and paper chromatography or retention time in the case of a column chromatographic technique. In this situation it would be less likely, although still possible, that an impurity would exhibit the same characteristic as one of the other species.

A few thin-layer systems of this type have been developed for ^{99m}Tc radiopharmaceuticals. Chiotellis et al. (1978) have shown that when the gall bladder imaging agent ^{99m}Tc-N-(2,6-dimethylphenylcarbamoylmethyl)-iminodiacetic acid (^{99m}Tc-HIDA) is analysed on ITLC-SG using 2% sodium chloride as the mobile phase, the ^{99m}Tc-HIDA migrates with an Rf of 0.5.

Ohta et al. (1984) have developed a system for analysis of pentavalent ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc(V)-DMSA) which uses a conventional silica gel thin-layer plate and a mobile phase of butanol: acetic acid: water (3:2:3). Under these conditions, ^{99m}Tc(V)-DMSA migrates with a Rf of 0.4 - 0.6, ^{99m}Tc-pertechnetate impurity migrates with an Rf of 0.65 - 0.8 while ^{99m}Tc(IV)-DMSA and unbound reduced ^{99m}Tc remain on the origin (Westera et al. 1985).

1.3 Electrophoresis

Billinghurst (1973) and Pauwels & Feitsma (1977) investigated electrophoresis as a technique for the routine analysis of ^{99m}Tc radiopharmaceuticals but concluded that it offered no advantages over the more rapid and easily performed thin-layer chromatographic techniques. Electrophoresis on cellulose acetate has been used by Baker et al. (1975) for the analysis of ^{99m}Tc-pyridoxylideneglutamate, a radiopharmaceutical for hepatobiliary imaging. However, this technique has not found widespread application. The slow speed of separation and poor reproducibility have prevented its adoption as a routine quality control technique for radiopharmaceuticals (Carpenter 1986).

1.4 Gel permeation chromatography

Gel permeation chromatography using Sephadex G25 (Pharmacia) has been suggested as a suitable technique for the analysis of ^{99m}Tc radiopharmaceuticals due to its ability to separate ^{99m}Tc-pertechnetate, labelled compound and unbound reduced ^{99m}Tc (Eckelman et al. 1971). The rationale for this technique is that the labelled compound is larger than the fractionation range of the gel and is therefore eluted at the void volume of the column. ^{99m}Tc-pertechnetate is smaller than the fractionation range and is therefore eluted at the total volume of the column. Unbound reduced ^{99m}Tc is irreversibly adsorbed onto the gel at the top of the column. Valk et al. (1973) have shown that when this technique is used to analyse the renal imaging radiopharmaceutical ^{99m}Tc-gluconate, the Sephadex gel can attract the ^{99m}Tc from the gluconate molecule thereby giving an erroneously high value of the level of unbound reduced ^{99m}Tc in the radiopharmaceutical. Steigman and Williams (1974) demonstrated that this phenomenon could be overcome by eluting the Sephadex column with a solution of the complexing compound rather than the 0.9% sodium chloride solution used in the technique described originally. As an alternative means of overcoming the problem, Billinghurst and

Palser (1974) suggested the use of Bio-Gel (Bio-Rad Laboratories) in place of Sephadex since, while it retains the ability to adsorb any unbound reduced ^{99m}Tc in the radiopharmaceutical, it does not have the power to strip ^{99m}Tc from the labelled compound.

Despite these findings, gel permeation chromatography which is practised in this way can be likened to the origin/solvent front approach of the miniaturized thin-layer techniques in that it is not specific for the labelled compound. Its popularity was short-lived , probably due to it being more technically demanding than the thin-layer techniques.

Gel permeation chromatography has been shown to be valuable in the analysis of labelled proteins and other large molecules. In these situations, it is necessary to choose a gel with a fractionation range that encompasses the sizes of the molecules to be separated. This has been shown to be valuable for the demonstration of ^{99m}Tc-labelled tin colloid impurity and albumin polymers in ^{99m}Tc-human albumin (Pettit et al. 1978 & 1980, Dekker et al. 1982). Gel permeation chromatography on a Sepharose-4B column has also been used to demonstrate the presence of a ^{99m}Tc-labelled colloidal impurity in ^{99m}Tc-labelled cardiac myosin antibody fragment (Khaw et al. 1982).

1.5 The case for high performance liquid chromatography

High performance liquid chromatography is used widely as an analytical technique for determining the purity of pharmaceuticals. In the field of radiopharmacy it has the potential to overcome the shortcomings of the popular but inadequate thin-layer techniques that have been described earlier. Most importantly, HPLC is a highly specific technique and should be capable of providing a peak with a specific retention time for each radiochemical species in a radiopharmaceutical. The analysis is carried out in an oxygen-free environment which should prevent degradation of the radiopharmaceutical during analysis. HPLC can be a rapid technique to perform and should, if necessary, allow measurement of radiochemical purity before the radiopharmaceutical is administered to the patient.

Early attempts to use HPLC for the analysis of ^{99m}Tc radiopharmaceuticals resulted in little more than sophisticated and expensive methods for the quantitation of ^{99m}Tc-pertechnetate impurity (Russell and Majerik, 1979, Wong et al., 1981). The reason for this inadequacy was the choice of chromatographic systems in which the radiopharmaceuticals were eluted from the HPLC columns as unretained solutes while any ^{99m}Tc-pertechnetate impurity was eluted

at some time later. This strategy is no different to the thin-layer origin/solvent front approach criticized earlier.

HPLC has been used widely during the development of new radiopharmaceuticals and in the investigation of the radiolabelled species that are present in new and existing radiopharmaceuticals.

The agents that have been most extensively investigated by HPLC are undoubtedly the bone imaging radiopharmaceuticals ^{99m}Tc-ethylenehydroxydiphosphonate (EHDP) and ^{99m}Tc-methylene diphosphonate (MDP). ^{99m}Tc radiopharmaceuticals have traditionally been thought of as discrete chemical entities but Srivastava et al. (1982) used a reversed phase HPLC column in conjunction with a complex eluent containing an ion-pairing agent to demonstrate that ^{99m}Tc-EHDP consists of eight major ^{99m}Tc-labelled species and ^{99m}Tc-MDP consists of four. More recently, Hoch and Pinkerton (1986) used a similar chromatographic system but with a macroporous reversed-phase column to demonstrate the presence of many more radiolabelled species in ^{99m}Tc-MDP. Analysis of ^{99m}Tc-EHDP and ^{99m}Tc-MDP has also been performed using an ion exchange column and a simple eluent consisting of 0.85M sodium acetate (pH 8.4) to reveal the multicomponent nature of these radiopharmaceuticals (Pinkerton et al., 1980, Tanabe et al., 1983). DeLigny and co-workers have also used HPLC to carry out extensive investigations of the nature, formulation and analysis of ^{99m}Tc-EHDP and ^{99m}Tc-MDP (DeGroot et al. 1985, 1986 & 1987, Gelsema et al. 1987, Huigen et al. 1988a & b, Nieuwland et al. 1989, Tji et al. 1990).

Loberg and Fields (1978) used HPLC on an octadecylsilyl (ODS) column with an acetonitrile/phosphate buffer mobile phase during work to establish the structure of the gall bladder imaging radiopharmaceutical ^{99m}Tc-HIDA. Fritzberg and Lewis (1980) used similar chromatographic conditions for a comparison of five ^{99m}Tc-iminodiacetate agents and an investigation of the behaviour of these radiopharmaceuticals in plasma and bile.

The effect of the presence of Tc99 during the preparation of ^{99m}Tc-dimercaptosuccinic acid, a radiopharmaceutical for kidney imaging, has been studied by Moretti et al. (1982) using an ODS column and phosphate buffer mobile phase.

High performance gel permeation chromatography has been used by Vallabhajosula et al. (1982), Hosain and Hosain (1982) and Muller (1985) to analyse the blood pool imaging radiopharmaceutical ^{99m}Tc-human albumin. These investigations have demonstrated the

presence of up to five radiolabelled species in this radiopharmaceutical. In previous work using conventional gel permeation chromatography, Pettit et al. (1978) succeeded in identifying only three components in ^{99m}Tc-HSA. The advantages of the HPGPC technique for the analysis of this radiopharmaceutical are therefore speed and higher resolution.

Blower et al. (1991) have used gradient elution of a styrene-divinylbenzene polymer column with an acetonitrile/trifluoroacetic acid mobile phase in the course of their work to determine the chemical identity of pentavalent ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc(V)-DMSA).

Using HPLC to obtain quantitative information on the composition of a radiopharmaceutical is not as straightforward as using thin-layer chromatography. As the solvent migrates up a thin-layer plate, all the radioactive material that was applied to the origin becomes distributed along the plate as one or more spots. During analysis of the chromatogram all the radioactive material that was applied to the plate is detected. This is not necessarily the case with HPLC. A fraction of the sample that is injected onto the column may become irreversibly bound to the column packing and remain undetected. If this adsorbed fraction is an impurity in the radiopharmaceutical, calculation of the radiochemical purity from the chromatogram will give an erroneously high result. Conversely, if the adsorbed fraction is the labelled species, the calculated radiochemical purity will be erroneously low. If HPLC is to be a valid method of determining radiochemical purity, measurement of the recovery from the column is essential to ensure that no adsorption has taken place.

A consistent feature of the above reports on the use of HPLC in the investigation of ^{99m}Tc radiopharmaceuticals is that recoveries from the columns are not quoted. While the techniques have undoubtedly been of value in determining the identity of radiolabelled species or demonstrating the multi-component nature of radiopharmaceuticals, they are not necessarily satisfactory techniques for the measurement of radiochemical purity since the question of adsorption of ^{99m}Tc onto the column has not been addressed.

1.6 Aims and objectives

The aim of this project was therefore to carry out a scientific investigation to establish if quantitative HPLC is a technique that can be used to measure the radiochemical purity of ^{99m}Tc radiopharmaceuticals.

The objectives to be met in achieving this aim were:

1. To develop, construct and evaluate an HPLC radiation detector for use with ^{99m}Tc radiopharmaceuticals.

2. To develop HPLC techniques for measuring the radiochemical purity of the radiopharmaceuticals ^{99m}Tc-mercaptoacetyltriglycine, ^{99m}Tc-dimercaptosuccinic acid and ^{99m}Tc-exametazime.

3. To evaluate the use of non-silica column packing materials in determining the radiochemical purity of these ^{99m}Tc radiopharmaceuticals.

4. To use the HPLC techniques to investigate aspects of the preparation of these ^{99m}Tc radiopharmaceuticals.

2. Equipment

A range of equipment was used to perform the experimental work that is described in the following chapters. Some of the instruments such as the HPLC pumps and nucleonics were commercially available while others such as the HPLC radiation detector and thin-layer/column scanner were developed for the project and constructed "in house". This chapter contains a description of each piece of equipment that was used. Throughout the text of this thesis I have restricted identification of suppliers to name only. Full names and addresses are contained in Appendix I.

2.1 HPLC pumps

Two pumps were used in the course of this work. The older was a Series 2/1 (Perkin-Elmer). This is a dual piston reciprocating pump capable of only isocratic operation. The newer of the two was a Model PU4100 (Philips Scientific). This is also a dual piston reciprocating pump and was chosen for its ability to perform single, isocratic and gradient elution. Its programmable operation was also a distinct advantage. The majority of the investigations were carried out with this instrument.

2.2 Injection valves

A Model 7120 (Rheodyne) injection valve was used in conjunction with the Perkin-Elmer pump and a Model 7125 injection valve (Rheodyne) was used in conjunction with the Philips PU4100 pump. Both valves were fitted with 20 μl sample loops.

2.3 HPLC columns

Hypersil-ODS (Shandon Scientific) is a fully capped octadecylsilyl adsorbent for reversed phase HPLC. A 250 x 5 mm analytical column was packed in-house with 5 μm adsorbent using a Shandon column packer. The efficiency of these columns was typically 50,000 plates per metre as measured with a phenol/para-cresol test mixture.

Hypercarb (Shandon Scientific) is an HPLC adsorbent that consists of rigid porous graphitised carbon spheres 7 μm in diameter. This was supplied in a 100 x 4.6 mm pre-packed column. The typical efficiency of the column as quoted by the manufacturer was 20,960 plates per metre. The potential advantages of this column are its high chemical resistance over a pH range of 1 to 14 and its freedom from the unreacted silanol sites that can be present in the conventional silica-based ODS reverse phase columns. These sites possess highly retentive properties and might therefore retain a significant proportion of the extremely low mass of radiolabelled compound that is present in a ^{99m}Tc radiopharmaceutical. Hypercarb is also interesting in that there is no documented report of its use for the analysis of ^{99m}Tc radiopharmaceuticals.

PLRP-S (Polymer Laboratories) is a spherical rigid macroporous polystyrene/ divinylbenzene HPLC packing. A 250 x 4.6 mm analytical column was used. This was supplied pre-packed with 8 μm adsorbent of 300 Å pore size. The analytical column was protected by a 5 x 3 mm PLRP-S guard column. The typical efficiency of this column as quoted by the manufacturer is 42,960 plates per metre. In common with Hypercarb, this column was chosen for its freedom from unreacted silanol sites and its stability over the pH range of 1 to 13.

PRP-1 (Hamilton) is a macroporous copolymer of styrene and divinylbenzene similar to PLRP-S. It was supplied as a pre-packed 150 x 4.1 mm analytical column. The 10 μm particle size adsorbent was used. The analytical column was protected by a 25 x 2.3 mm PRP-1 guard column. The typical efficiency of this column as quoted by the manufacturer is 31,020 plates per metre. In common with Hypercarb and PLRP-S, this adsorbent is free from unreacted silanol sites and is stable over the pH range of 1 to 14.

Injector-to-column and guard column-to-analytical column connections were made using tubing with an internal diameter of 0.3 mm.

2.4 Radiation detector

The gamma radiation detector was constructed from a length of stainless-steel column outlet tubing (internal diameter = 0.3 mm) positioned inside or across the face of a 5 x 5 cm sodium iodide crystal detector with a 1.5 x 4 cm well. Typical configurations of tubing and crystal are shown in Figure 2.1. The different arrangements gave flow-cell volumes of between 2 and 10 μl and were used to achieve different sensitivities of detection. Configurations A, B and C gave relative sensitivities of 1.5, 1.0 and 3.6 respectively. In configuration A, replacement of the stainless-steel tubing with polyether-etherketone (PEEK) tubing resulted in a 30% increase in detection sensitivity due to the lower attenuation of gamma radiation in PEEK. The whole assembly was surrounded by lead shielding. Two nucleonic systems were used to create the chromatograms from the output of this detector.

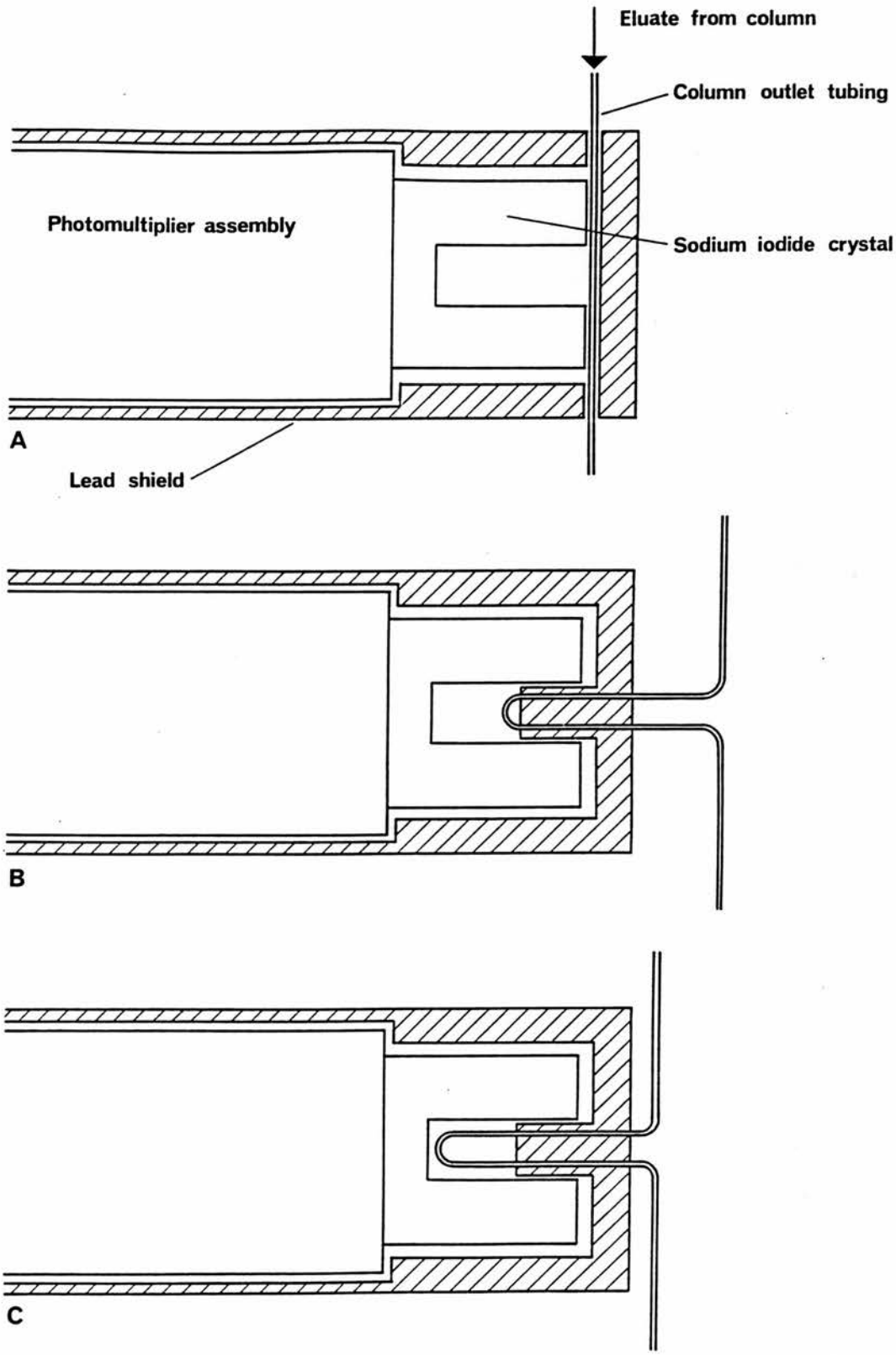

Figure 2.1. *Diagrammatic illustrations of detector cell configurations.*

2.5 MS310 counter-ratemeter system

This was the older of the two systems. The signal from the detector was taken to a counter ratemeter Model MS310 (J & P Engineering) which also provided the high voltage supply to the detector. The analogue output from the MS310 counter-ratemeter was taken to a chart recorder Model E22 (Linseis) to provide chromatograms. The digital output from the MS310 was taken to a printer Model 412L (Datac) via a print controller module Model MS190 (J & P Engineering) to record the counts detected. When a counting time was set on the MS310 and counting was started, the instrument counted for the set time, the counts were printed, the display of the instrument was reset to zero and counting was restarted automatically. This process resulted in a listing of the counts detected against time. From this listing, the group of counts corresponding to a peak on the chromatogram could be identified and summed to give the total counts in the peak.

2.6 Accuspec system

In this, the newer of the two systems, the signal from the detector was taken to a SpecMate preamplifier/amplifier (Canberra Nuclear Data). The SpecMate also supplied the high voltage to the photomultiplier of the detector.

The output from the SpecMate was taken to an AccuSpec multichannel analyser (Canberra Nuclear Data). The AccuSpec system consists of both hardware and software components which are installed in a personal computer (PC). The hardware consists of a PS2/2 Model 30 PC (IBM) with the Accuspec FMS (Fast Multichannel Scaling) acquisition board installed in one of the computer's input/output channel slots. The software consists of the Accuspec Display and Acquisition software version 4.0 which was run under the Microsoft Disk Operating System (MS-DOS) version 4.01. The multichannel analyser was used in multiscaling mode to record chromatograms which were then stored on the computer's hard disk for subsequent analysis, manipulation and print-out. Chromatograms were produced by using an Accuspec utility to convert the data into Lotus 1-2-3 format, transferring it to SigmaPlot 4.1 software (Jandel) and printing with a LaserJet 4 printer (Hewlett Packard).

A diagrammatic illustration of the equipment is shown in Figure 2.2

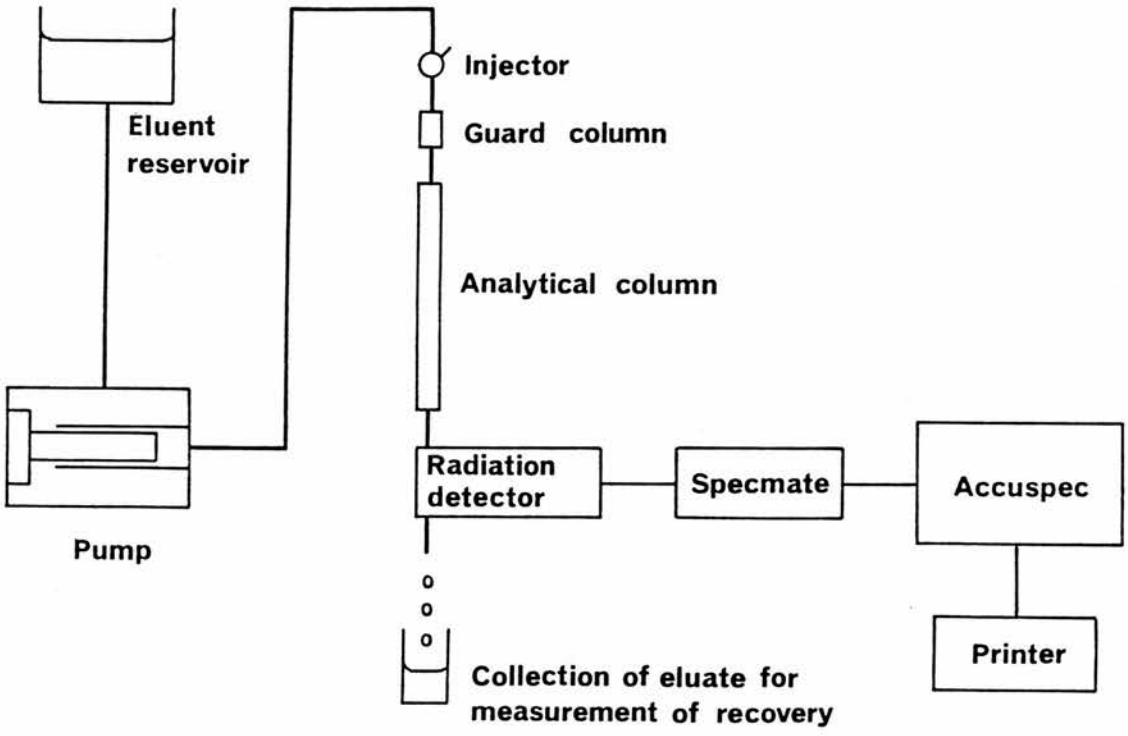

Figure 2.2. *Diagrammatic illustration of HPLC equipment.*

2.7 Measurement of detector dead-time

Multichannel analysers are notorious for suffering from count loss due to dead time at relatively low count-rates. In HPLC, peaks on the chromatogram can be extremely sharp. Count-rates can therefore be very high for short periods and any equipment used as a radiation detector for HPLC must be capable of handling these high count-rates without suffering loss of counts. To measure the count loss due to dead-time in the Accuspec system, a ^{99m}Tc source which would give a count-rate of approximately 30,000 counts per second (cps) was prepared and placed in the detector. The counting window of the Accuspec was set up for the 140 keV gamma-ray of ^{99m}Tc. The source was counted using a dwell time of 60 seconds for all 4096 channels. This gave a total counting time of 4096 minutes which represents 11.3 half-lives for ^{99m}Tc. The source was then removed from the detector and background counts were recorded for 70 minutes. An average background count per channel

was calculated and subtracted from the ^{99m}Tc counts detected in each of the 4096 channels. On the assumption that dead-time was insignificant up to 5,000 cps, the numbers of the channels containing count-rates between 250 and 5000 cps were noted at 250 cps intervals. The channel numbers were then plotted against the logarithm of the recorded count-rates. Using a linear regression, the equation for the line was obtained. This equation was consistent with a half-life of 6.02 hours. The correlation between channel number and logarithm of the count-rate ($r = 1.00$) justified the assumption that dead time is insignificant at count-rates up to 5,000 cps. Using the equation, the numbers of the channels which would contain count-rates between 1,000 and 30,000 cps were calculated at 1,000 cps intervals. The count-rates observed in these channels were noted. These observed count-rates were plotted against the true count-rates. This plot is shown in Figure 2.3. At 10,000, 20,000 and 30,000 cps, the losses due to dead-time were shown to be 2, 6 and 9% respectively.

The highest count-rate detected in a chromatogram obtained in the course of this work was 6,000 counts per second. At this count-rate, the loss due to dead-time was 0.5%. At no time, therefore, was loss of counts due to dead time a significant factor.

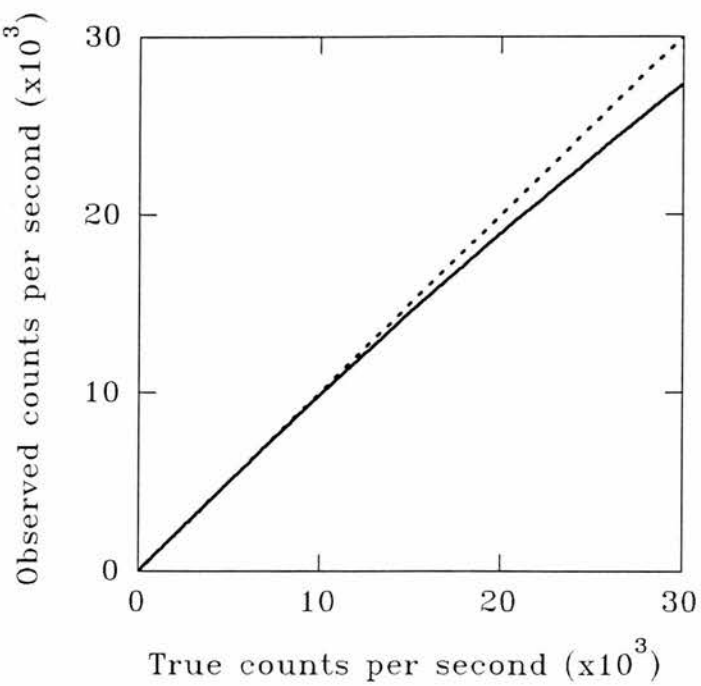

Figure 2.3 *Effect of dead-time on the efficiency of the radiation detector.*

2.8 Detector for measuring count-rates from column eluates

To determine the recoveries from HPLC columns, it was necessary to measure the count-rates from column eluates and aliquots of the samples being analysed. Given that the samples injected onto the columns were 20μl aliquots of radiopharmaceuticals containing between 50 and 400 MBq of ^{99m}Tc per ml, eluates could contain up to 8 MBq. This activity is too high to be counted in a conventional gamma-counter with a sodium iodide crystal detector. These eluates were therefore counted 15 cm above a detector of this type. This was achieved by using a platform which was fitted to the lead shielding around the detector. The platform was marked to enable samples to be positioned reproducibly above the detector. The arrangement of the detector and platform are shown in Figure 2.4. The detector was connected to a counter-ratemeter Model SR3 (Nuclear Enterprises).

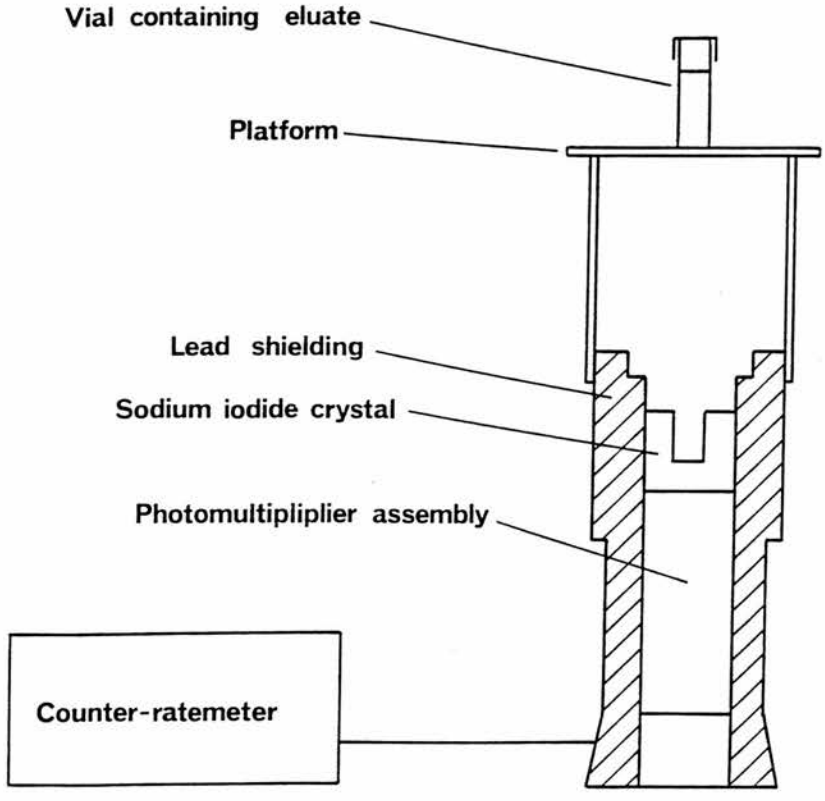

Figure 2.4 *Radiation detector configuration for counting recovery samples.*

2.9 Thin-layer and paper chromatogram scanner

In experiments that involved the analysis of radiopharmaceuticals by thin-layer and paper chromatography, the distribution of activity on the plate or paper strip was measured using a radiochromatogram scanner. This instrument was constructed by positioning a shielded sodium iodide crystal detector above a moving table provided by a chart recorder. A slit collimator was placed between the detector and the table with the slit at right angles to the direction of travel of the table. A slit width of 2 mm was used throughout the experiments. The collimator was positioned 5 mm above the bed. The detector was connected to the Specmate/Accuspec system described previously. A diagram of the scanner is shown in Figure 2.5.

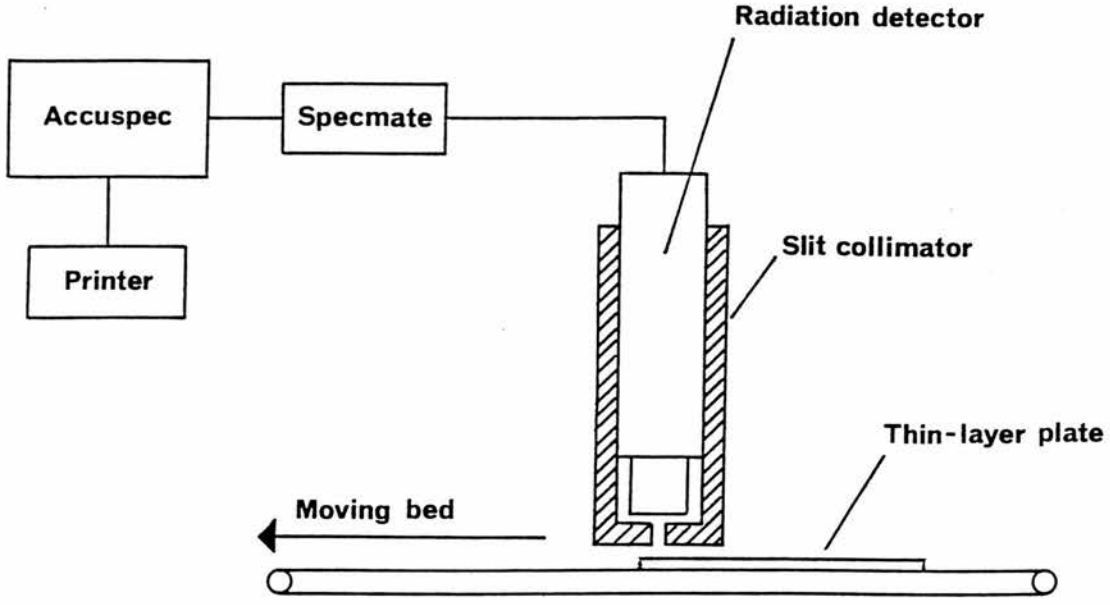

Figure 2.5 *Diagrammatic illustration of thin-layer and paper radiochromatogram scanner.*

2.10 HPLC column scanner

To locate the position of ^{99m}Tc retained in an HPLC column, the column was scanned using a modification of the thin-layer/paper radiochromatogram scanner. Whereas in the configuration for scanning thin-layer and paper chromatograms the collimator was positioned 5 mm above the bed, in this modified equipment for HPLC columns, the distance between the collimator and bed was increased to 20 mm to allow passage of the column through the instrument. As before, the detector was connected to the Specmate/Accuspec system described previously. A diagram of the scanner is shown in Figure 2.6.

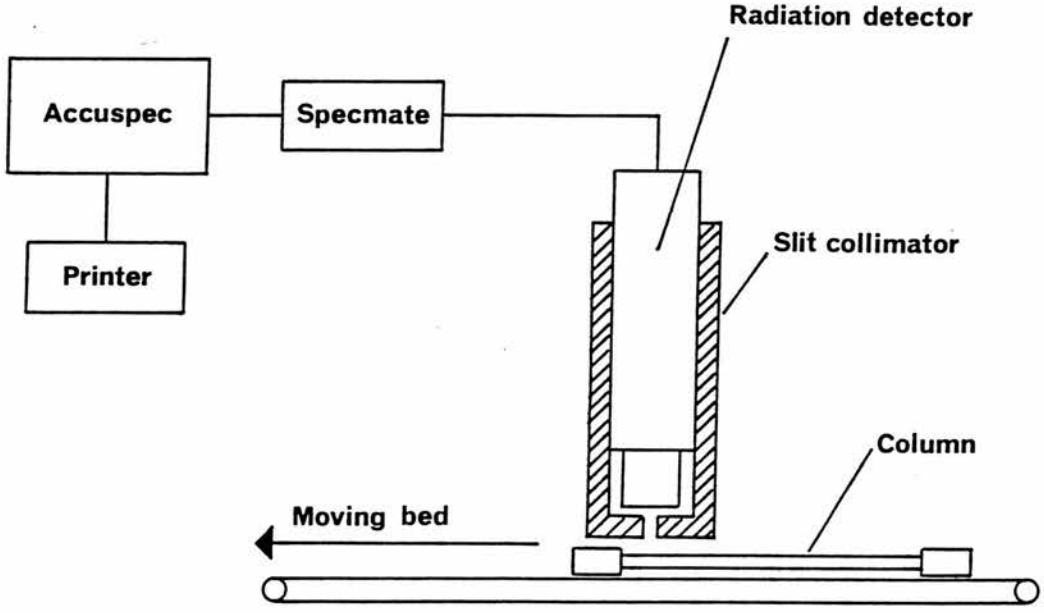

Figure 2.6 *Diagrammatic illustration of scanner for HPLC columns.*

3. Methods

A number of techniques were used throughout the course of my investigations. Those that were common to more than one investigation are described in this chapter. Those that were specific to a particular investigation, e.g. the technique used to prepare a radiopharmaceutical, are described in the chapter dealing with that aspect of the work.

3.1 Preparation of mobile phases

Mobile phases were prepared from HPLC grade solvents and chemicals. Water to the standard of Water For Injections B.P. was used in the preparation of aqueous components. Mobile phases were filtered through glass fibre filters with a pore size of 0.2 μm (Whatman) to remove particulate contamination and then degassed by sparging with helium for 5 minutes.

3.2 Sample injection technique

The introduction of the sample onto the HPLC column was performed according to the conventional complete loop filling technique described by Rheodyne. A potential drawback of this technique is that it requires an excess of sample to be available. However, this does not present a problem with ^{99m}Tc radiopharmaceuticals since they tend to be prepared in volumes of a few millilitres. Adequate sample is therefore available. Apart from its ease of execution, the advantage of the complete loop filling technique is that it delivers the highly reproducible sample volumes that are required if recovery from the HPLC column is to be measured by collection and counting of the activity of the eluate. Sample injection was carried out as follows. With the valve in the "Load" position and mobile phase being pumped through the valve to the column, water was injected into the needle port to flush the sample loop. At the end of this procedure the loop contained water. Approximately 300 μl of the radiopharmaceutical to be analysed was drawn into a 1 ml disposable syringe. Approximately 200 μl of air was drawn into the syringe which was then held upright and tapped to bring all the solution to the plunger. The 22 gauge sample needle supplied with the valve was fitted to the syringe and inserted into the needle port of the injection valve. The sample was discharged slowly from the syringe. This had the effect of expelling the

flushing water from the sample loop then passing the air in the syringe through the loop and finally filling the loop with sample. The discharge from the loop vent tube was directed into a vial which was contained inside a lead shield. This arrangement was used to prevent the discharged radioactive liquid from increasing the background radiation in the vicinity of the detector. Passing 200 μl of air through the loop ahead of the sample prevented mixing of sample and flushing water which might have resulted in dilution of the sample. Immediately after filling the loop, the valve was switched to the "Inject" position, the acquisition of the chromatogram was started and the sample syringe was removed from the valve, again to minimise the background radiation in the vicinity of the detector. The valve was left in the "Inject" position until the end of the analysis.

3.3 Recording of chromatograms from HPLC

The high voltage and gain of the Specmate pre-amplifier/amplifier and the energy window of the Accuspec multichannel analyser were set to detect the 140 keV gamma ray emitted by ^{99m}Tc. The dwell time of the Accuspec was set to a suitable time - this was typically 2 seconds per channel. Immediately after injection of the sample onto the column, the Accuspec was switched on and the counts from the detector were acquired until the final peak had eluted from the column. At the end of acquisition, the data were saved on the hard disk of the computer. A printed chromatogram was obtained by manipulating the data using SigmaPlot (Jandel) plotting software.

A similar procedure was adopted when using the older detector system based on the MS310 counter-ratemeter which produced a chromatogram on a chart recorder and a print-out of the counts detected in each counting interval.

3.4 Measurement of recovery from HPLC columns

When using HPLC for determination of the radiochemical purity of a radiopharmaceutical, it is possible that a fraction of the sample injected onto the column may become irreversibly bound to the column packing and remain undetected. If this adsorbed fraction is an impurity in the radiopharmaceutical, calculation of the radiochemical purity using the counts from the radiation detector will give an erroneously high result. Conversely, if the adsorbed fraction is labelled compound, an erroneously low radiochemical purity will be calculated. It is

important therefore to measure the recovery from the column to ensure that no adsorption has taken place. This can be achieved by injecting a known volume of sample onto the column, collecting the eluate and comparing the count-rate from it with the count-rate from a standard prepared by pipetting an identical volume of the sample. If the count-rates from sample and standard are identical, recovery is 100%. There is, however, a drawback to this technique. Samples were injected onto the columns using loop-valve injectors fitted with 20 μl loops. This is known to be a highly reproducible method of applying samples to HPLC columns. However, Rheodyne, the manufacturer of the valves, acknowledges that the volume quoted for a sample loop is only nominal and can vary from the actual volume by as much as 20%. This is due to the $\pm$ 0.001" tolerance on the internal diameter of the tubing. The 20 μl loops used in my work are made from tubing of 0.02" bore. Since the volume tolerance on these loops is therefore $\pm$ 10%, there was every likelihood that the nominal 20 μl loop would not deliver the same volume as a 20 μl pipette. It was necessary therefore to calibrate one against the other. To do this, 25 x 20 μl aliquots of a Sodium Pertechnetate [^{99m}Tc] Injection were pipetted into vials using a 20 μl fixed volume capillary pipette (SMI). With no column fitted to the Rheodyne valve and using water as the mobile phase, 25 loopfuls of the same ^{99m}Tc solution were delivered directly into vials containing approximately 15 ml of water. During this process, the outlet tubing from the valve was positioned below the surface of the water. This was done to ensure that no sample was lost as spray when the valve was switched from the "Load" to the "Inject" position and the system was momentarily put under pressure. The volume in each vial was made up to 20 ml and the solution was mixed thoroughly. Using the equipment described in 2.8, the count-rate from each of the 50 vials was measured. The counting time was chosen to result in the detection of a minimum of 10,000 counts to minimize the effect of statistical variation that results from the random nature of radioactive decay. "Pipette" and "loop" samples were counted alternately to minimize the effect of any possible instability in the counting equipment. A record was made of the time at which each vial was measured and after subtraction of background counts, the counts detected were decay-corrected back to the time of measurement of the first vial. The average counts from the "loop" samples were divided by the average counts from the "pipette" samples to give the correction factor.

As part of the analysis of a radiopharmaceutical by HPLC, the recovery of the sample from the column was measured as follows. After injection of a sample of the radiopharmaceutical onto the column, the eluate was collected in a vial. At the end of the collection period, i.e. when the radiation detector was registering only background counts from the eluate, the

vial was removed from the instrument and its contents were thoroughly mixed. Aliquots of the radiopharmaceutical were pipetted into three identical vials using the 20 μl pipette. The 20 μl samples were diluted to a volume equal to the volume of eluate collected and mixed thoroughly. The count-rate from the solution in each of the four vials was measured using the equipment described in 2.8. As above, a counting time that resulted in the detection of at least 10,000 counts was chosen. After subtraction of background counts and correction for the discrepancy between the volumes of loop and pipette, recovery from the column was calculated by expressing the count-rate from the eluate as a percentage of the mean count-rate from the three standards.

To establish the range within which a measurement of recovery could be considered satisfactory, the error associated with the technique was determined by measuring the error in each step of the procedure. The error associated with delivery of the sample from the Rheodyne valve was measured by the following technique. With no column attached to the valve, a 20 μl sample of a sodium pertechnetate [^{99m}Tc] solution was delivered into each of 25 universal containers. As in the earlier experiment to calibrate the valve against the pipette, the valve outlet tube was positioned under water in the universal containers during delivery of the samples. The solution in each container was made up to 20 ml with water, mixed thoroughly and counted using the equipment described in 2.8. Counting was performed for a time that resulted in the acquisition of at least 50,000 counts as this is typical of the total counts acquired during a recovery measurement. A record was made of the time at which each vial was counted. The counts detected were then corrected for the decay since the time at which the first vial was counted. The coefficient of variation for delivery from the valve was found to be 1.0%. The error associated with pipetting of the standards was measured in a similar manner. A 20 μl sample of a sodium pertechnetate [^{99m}Tc] solution was pipetted into each of 25 universal containers. The samples were diluted to 20 ml, mixed and counted. The coefficient of variation for pipetting was found to be 2.3%. In the technique for measurement of recovery, the standards are prepared and counted in triplicate. The coefficient of variation must therefore be reduced by a factor of $\sqrt{3}$. The error associated with positioning of the containers on the platform over the detector was determined by counting one universal container of ^{99m}Tc 25 times, the vial being removed and replaced between each measurement. Corrections for decay were applied as before and the coefficient of variation for this part of the procedure was found to be 0.6%. As before, it is necessary to reduce this repositioning error for the standards by a factor of $\sqrt{3}$. The overall coefficient of variation for the recovery technique was then calculated as the square root of the sum of the squares of the individual errors and found to

be 1.8%. With a 95% level of confidence, therefore, a measurement of recovery that falls within two coefficients of variation of 100%, i.e. between 96.4% and 103.6%, can be considered satisfactory.

3.5 Quantitative analysis of chromatograms

Quantitative analysis was undertaken to determine the percentage of the total activity of the radiopharmaceutical that is represented by each peak in the chromatogram. At the start of the chromatogram, the counts in a number of channels were used to calculate the mean background counts per channel. This was typically calculated from the counts in the first 10 channels. With none of the columns used in the course of this work was there any possibility of a compound being eluted within 20 seconds. The start of the first peak was taken as the channel in which the count-rate increased in relation to the background. The end of the peak was taken as the channel after which the count-rate either returned to background or began to increase due to the elution of a second species. Using these criteria, the start and end of each peak were determined. One of the Accuspec's features is the facility to include all the channels between two markers in a region of interest from which the software calculates and displays the number of counts in the region. By using this feature to place regions around the relevant parts of the chromatogram, the counts in each peak were determined. Based on the number of channels that constituted each peak, the background counts in each peak were calculated from the mean background counts per channel established at the start of the chromatogram. The background counts were then subtracted from the counts in each peak. The percentage of the total activity contained in each peak was then calculated by expressing the counts in the peak as a percentage of the total counts in the chromatogram.

When using the MS310 detector system, identification of the peaks on the print-out was performed according to the criteria described above. Calculation of the counts in each peak was carried out manually by addition of the counts in the counting intervals that had been deemed to form the peak. The subsequent background correction and calculation of the percentage of total activity in each peak was carried out as described above.

The times taken to record chromatograms were sufficiently short to obviate the need for correction of the counts in each channel for the decay that had occurred since the start of the data acquisition.

3.6 HPLC column scanning

When measurement of recovery from the HPLC column indicated that a proportion of the sample had been retained, the column was removed from the instrument and scanned using the equipment shown in Figure 2.6. The collimator slit was set at 2 mm and the column was moved past the detector at a speed of 1 cm/minute. Scanning was started 3 cm before the top of the column and ended 3 cm after the bottom of the column had passed the detector. The profile of the ^{99m}Tc retained in the column was recorded using the Accuspec multichannel analyser. A print-out of the profile of activity was produced using the SigmaPlot software as described previously. The use of scanning for quantitation of ^{99m}Tc retained on a column was not attempted since the stainless-steel fittings result in different thicknesses of metal between the column packing and the detector at various points along the column. This results in varying absorption of the radiation along the length of the column and makes quantitation impossible. Scanning was therefore used only to localize retained ^{99m}Tc.

3.7 Paper and thin-layer plate scanning

Scanning of paper chromatograms and thin-layer plates was performed with the same equipment as was used to scan columns but with the detector positioned only 5 mm above the moving table (Figure 2.5). During scanning, paper strips and TLC plates with an aluminium sheet support were covered with a sheet of glass (2 mm thick). This resulted in a 17% reduction in count-rate due to absorption of the gamma radiation in the glass but was done to keep all parts of the plate equidistant from the detector and thereby ensure a consistent efficiency of detection along the length of the plate. Due to their inherent rigidity, thin-layer plates on a glass support did not have to be treated in this manner. Scanning was started 3 cm below the origin of the chromatogram and ended 3 cm above the solvent front. The counts detected from the plate were recorded using the Accuspec multichannel analyser. The chromatogram was obtained by plotting the data with the SigmaPlot software as described previously.

3.8 Statistical analysis

Statistical analyses were performed using the Unistat-II statistical software (Unisoft) on a BBC Master Series microcomputer (Acorn Computers).

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

The work described in this chapter was undertaken to establish the suitability of HPLC for determination of the radiochemical purity of Technetium-99m Mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$) and then to use the HPLC technique to measure the radiochemical purity of ^{99m}Tc-MAG$_3$ prepared under a variety of conditions that might arise in routine radiopharmacy practice.

4.1 Introduction

^{99m}Tc-MAG$_3$ is a relatively new radiopharmaceutical that has been developed for gamma-camera renography (Fritzberg et al. 1986). A number of publications have shown ^{99m}Tc-MAG$_3$ to be a suitable replacement for ^{123}I- and ^{131}I-iodohippurate and ^{99m}Tc-diethyltriaminepentaacetic acid (^{99m}Tc-DTPA) in the investigation of renal function (Taylor et al. 1986, 1987, 1988, Bubeck et al. 1988, Jafri et al. 1988, Russell et al. 1988, Al-Nahhas et al. 1988). ^{99m}Tc-MAG$_3$ is now in widespread clinical use. A proposed structure for the ^{99m}Tc-MAG$_3$ complex is shown in Figure 4.1 (Fritzberg et al. 1986).

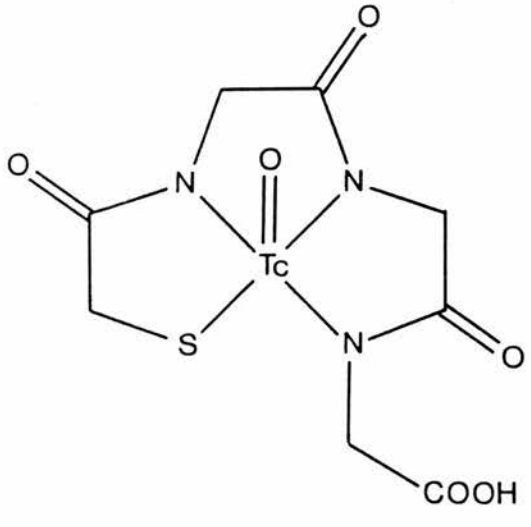

Figure 4.1 *Proposed structure for ^{99m}Tc-mercaptoacetyltriglycine (MAG$_3$)*

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

In response to the encouraging clinical results with this radiopharmaceutical, Mallinckrodt Medical introduced Technescan MAG$_3$, a kit for the preparation of ^{99m}Tc-MAG$_3$, to the U.K. market in 1988. This kit contains 1 mg of betiatide (benzoylmercapto-acetyltriglycine), 16.9 mg of disodium tartrate and 40 μg of stannous chloride. Preparation of ^{99m}Tc-MAG$_3$ involves reconstituting the kit with Sodium Pertechnetate [^{99m}Tc] Injection then incubating the vial in a boiling water bath for 10 minutes. Upon addition of the Sodium Pertechnetate [^{99m}Tc] Injection to the kit, a ^{99m}Tc-tartrate complex is formed. During heating, the benzoyl moeity is cleaved from the betiatide and exchange of Tc occurs between the tartrate and MAG$_3$ molecules resulting in the formation of the ^{99m}Tc-MAG$_3$ complex.

Thin-layer chromatography, paper chromatography and HPLC have all been used for the analysis of ^{99m}Tc-MAG$_3$. Mallinckrodt (personal communication) has proposed methods for determining the radiochemical purity of ^{99m}Tc-MAG$_3$ that employ each of the three techniques. Chen et al. (1993) have proposed a thin-layer technique that involves the use of two plates developed with different solvents. This report included a favourable comparison with an HPLC technique. In several studies of ^{99m}Tc-MAG$_3$, HPLC has been used to demonstrate that a single radiochemical species predominates in the final product of the radiolabelling procedure (Fritzberg et al. 1986, Coveney & Robbins 1987, Schaap et al. 1988, Taylor et al. 1988, Brandau et al.1988). However, none of these reports contain confirmation that HPLC is a satisfactory technique for measuring the radiochemical purity of ^{99m}Tc-MAG$_3$. In particular, none of the HPLC analyses included a measurement of recovery from the HPLC column. The work described in this chapter was therefore undertaken to:

1. develop and validate an HPLC technique for measurement of the radiochemical purity of ^{99m}Tc-MAG$_3$

2. use HPLC to measure the radiochemical purity and stability of ^{99m}Tc-MAG$_3$ that has been prepared according to several different protocols

4.2 Comparison of thin-layer, paper and high performance liquid chromatography

^{99m}Tc-MAG$_3$ was prepared according to the following method recommended by Mallinckrodt. Sodium Pertechnetate [^{99m}Tc] Injection was obtained from a ^{99m}Tc generator (Product code MCC20, Amersham), diluted to a radioactive concentration of

500 MBq/10 ml with Sodium Chloride Injection and injected into a MAG_3 kit (Product code DRN 4334, Mallinckrodt). The vial was placed in a boiling water-bath for 10 minutes then transferred to a beaker of water at room temperature for 10 minutes. The final product was stored at room temperature.

Two impurities that might be anticipated in ^{99m}Tc-MAG_3 are ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate. ^{99m}Tc-pertechnetate could be present due to either insufficient reducing agent in the MAG_3 kit or oxidation of the ^{99m}Tc-MAG_3 after preparation. ^{99m}Tc-tartrate could be present due to incomplete exchange of ^{99m}Tc between the tartrate complex and MAG_3. To investigate the chromatographic behaviour of these potential impurities and determine the ability of each chromatographic system to separate them from ^{99m}Tc-MAG_3, samples of each were prepared. ^{99m}Tc-pertechnetate was readily available from a ^{99m}Tc generator. ^{99m}Tc-tartrate was prepared by the following technique. Tartaric Acid 4.23 g then stannous chloride dihydrate 10 mg were dissolved in 100 ml of Water for Injection which had been purged with nitrogen for 10 minutes - this is the ratio in which tartaric acid and stannous chloride are present in the MAG_3 kit. A 1.0 ml aliquot of this solution was transferred to a 10 ml vial containing a nitrogen atmosphere and Sodium Pertechnetate [^{99m}Tc] Injection (500 MBq/9 ml) was injected into the vial.

Thin-layer chromatography was performed on 5 x 10 cm reversed phase plates (RP18, Product Code 15685, Merck) as recommended by Mallinckrodt. Immediately before use, the plates were dried at 120°C for 15 minutes then cooled. The origin was marked 1.5 cm from the bottom of the plate. The solvent front was marked 6 cm above the origin. Six spots of the radiopharmaceutical to be analysed were applied to the origin with a 1 ml syringe fitted with a 25G hypodermic needle. The spots were dried under a stream of nitrogen before the plate was placed in a chromatography tank containing a solvent of 0.9% aqueous sodium chloride/methanol/acetic acid (55:45:1). Once the solvent had migrated the required distance, the plate was removed from the tank and dried under hot air. The plate was scanned at 1 cm/minute using the thin-layer scanner described in Chapter 2. The chromatogram was recorded in the Accuspec using a dwell time of 5 seconds. Typical chromatograms from ^{99m}Tc-MAG_3, ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate are shown in Figure 4.2 and contain peaks with Rf values of 0.60, 0.90 and 0.90 respectively.

Paper chromatography was performed on 4 x 18 cm strips of 3MM paper (Whatman) as recommended by Mallinckrodt. The origin was marked 2 cm from the bottom of the strip. The solvent front was marked 10 cm above the origin. The solvent was run in the machine

direction of the paper. The sample was applied and dried in the manner described above. The strip was then placed in a chromatography tank containing acetonitrile/water (60:40). Once the solvent had travelled the marked distance, the strip was removed from the tank, dried and scanned as above. A dwell time of 10 seconds was used. Typical chromatograms from ^{99m}Tc-MAG$_3$, ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate are shown in Figure 4.3 and contain peaks with Rf values of 0.55, 0.90 and 0.90 respectively.

HPLC was performed using a modification of the method provided by Mallinckrodt. The equipment used for this analysis consisted of the Philips pump fitted with the Rheodyne 7125 injection valve, a 250 x 5 mm Hypersil-ODS column and the Accuspec-based radiation detector with the detection cell in configuration C (Figure 2.1). The column was eluted at 1 ml/minute with ethanol/10 mM phosphate buffer pH6 (5:95) for 7 minutes then the pump switched to methanol/water (90:10) for 10 minutes. The recovery from the column was measured according to the method described in Chapter 3.4. Typical chromatograms from ^{99m}Tc-MAG$_3$, ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate are shown in Figure 4.4 and contain peaks with retention times of 4.6, 2.8 and 2.1 minutes respectively.

The chromatograms demonstrate that each of the three techniques separates ^{99m}Tc-MAG$_3$ from the impurities ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate. None of the techniques separate the impurities although HPLC did produce a chromatogram containing two peaks. The coincidence of the ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate peaks on the paper and thin-layer chromatography could be the result of no radiolabelling having been achieved in the preparation of ^{99m}Tc-tartrate, the ^{99m}Tc remaining in the form of ^{99m}Tc-pertechnetate and thereby giving identical chromatograms. However, the two peaks in the HPLC chromatogram suggest that some radiolabelling did occur. It is possible that the first peak is due to ^{99m}Tc-tartrate while the second is due to ^{99m}Tc-pertechnetate that is present as an impurity. To determine if ^{99m}Tc-pertechnetate was present in the ^{99m}Tc-tartrate, the solution of ^{99m}Tc-tartrate was analysed by a miniaturized thin-layer technique. This was performed using an Instant Thin Layer Chromatography (ITLC/SG, Gelman) plate with butan-1-one as the solvent. Under these conditions, ^{99m}Tc-pertechnetate is known to migrate with the solvent front while radiolabelled species remain at the origin. Analysis of the ^{99m}Tc-tartrate solution demonstrated 8% of the activity at the solvent front, presumably due to the presence of ^{99m}Tc-pertechnetate. The 92% of the ^{99m}Tc that remained at the origin demonstrates that a reasonably high degree of radiolabelling was achieved. It can therefore be concluded that ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate behave similarly on paper and thin-layer chromatography but not on HPLC.

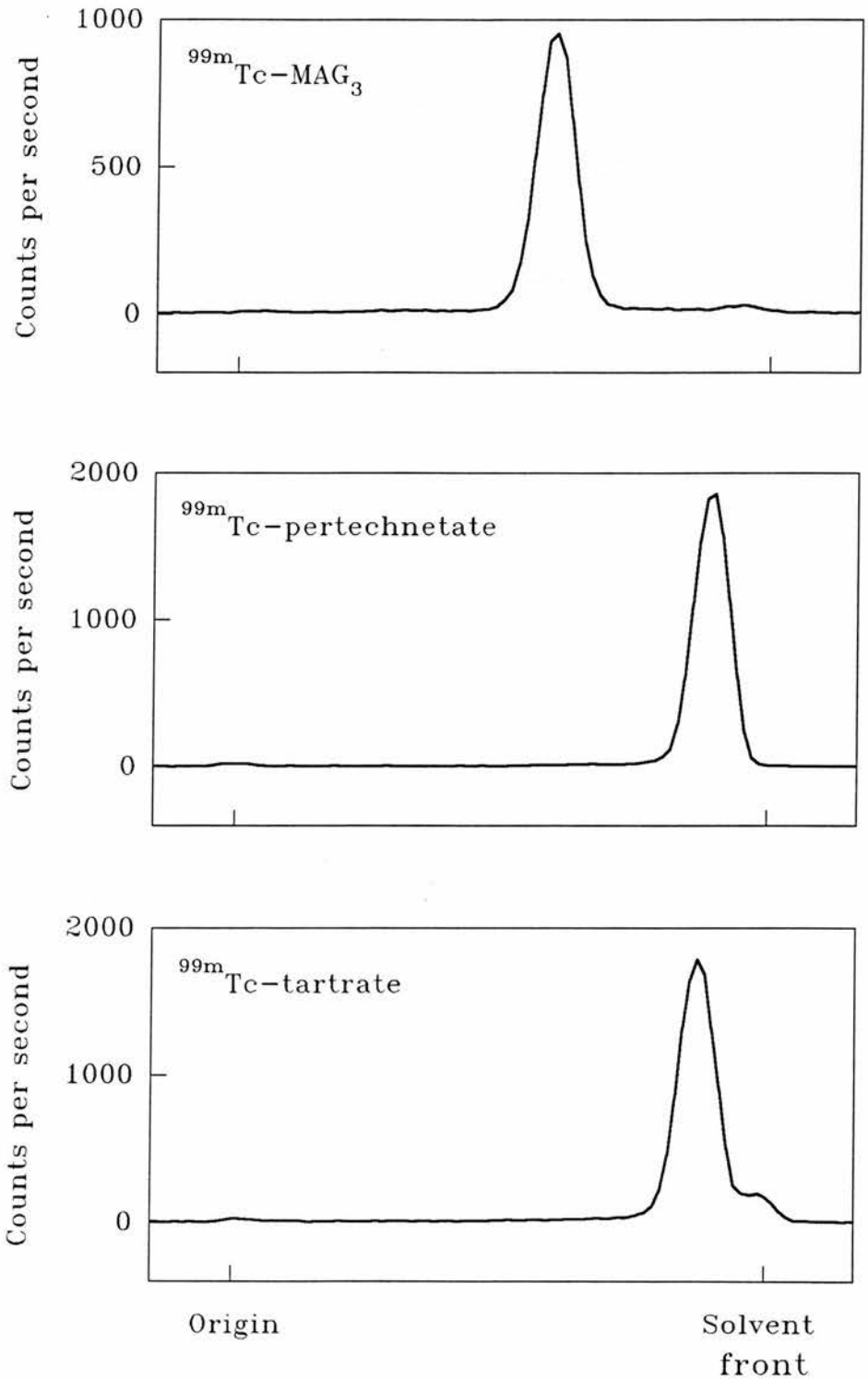

Figure 4.2 *Thin-layer chromatograms of ^{99m}Tc-MAG$_3$, ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate.*

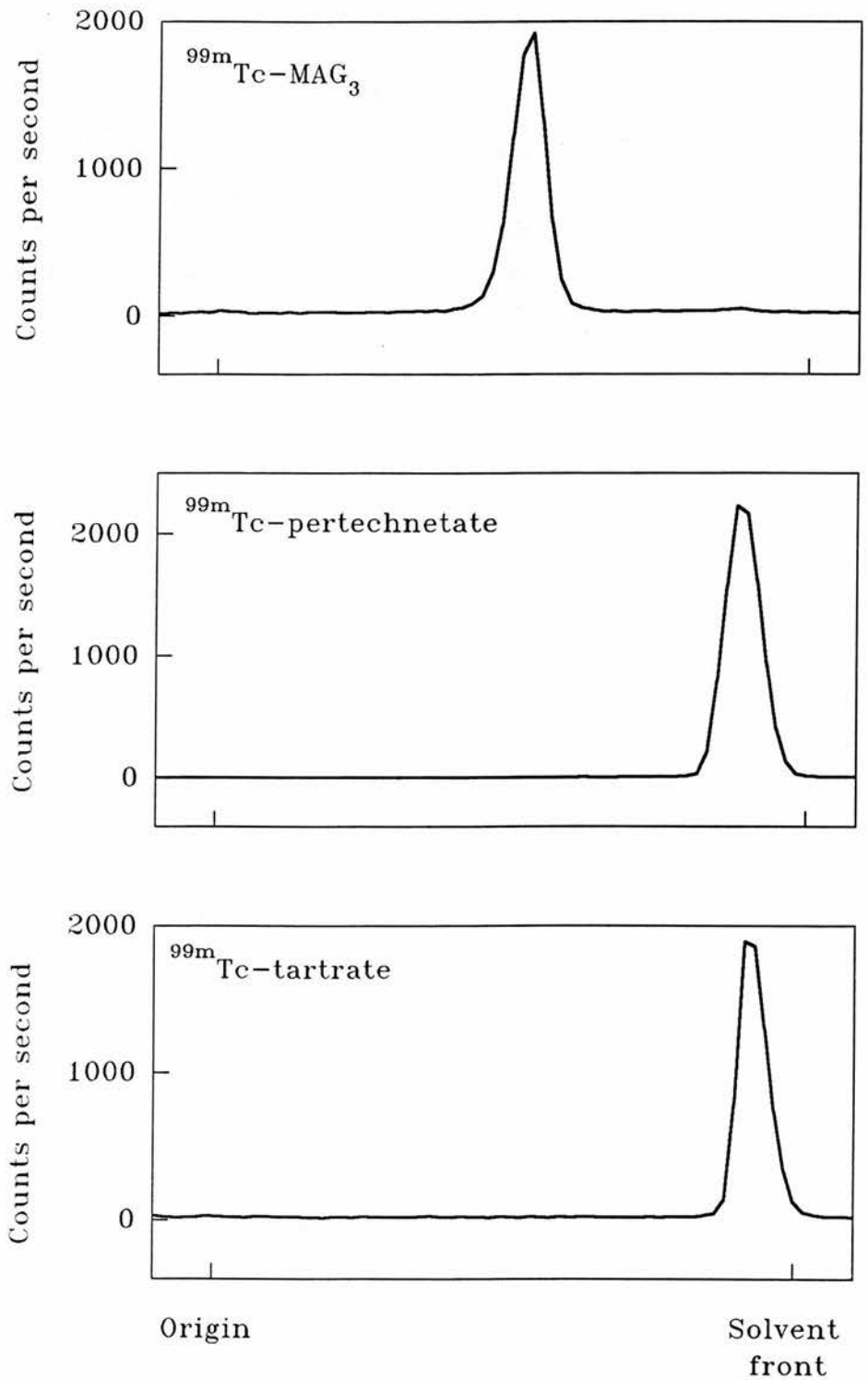

Figure 4.3 *Paper chromatograms of ^{99m}Tc-MAG$_3$, ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate.*

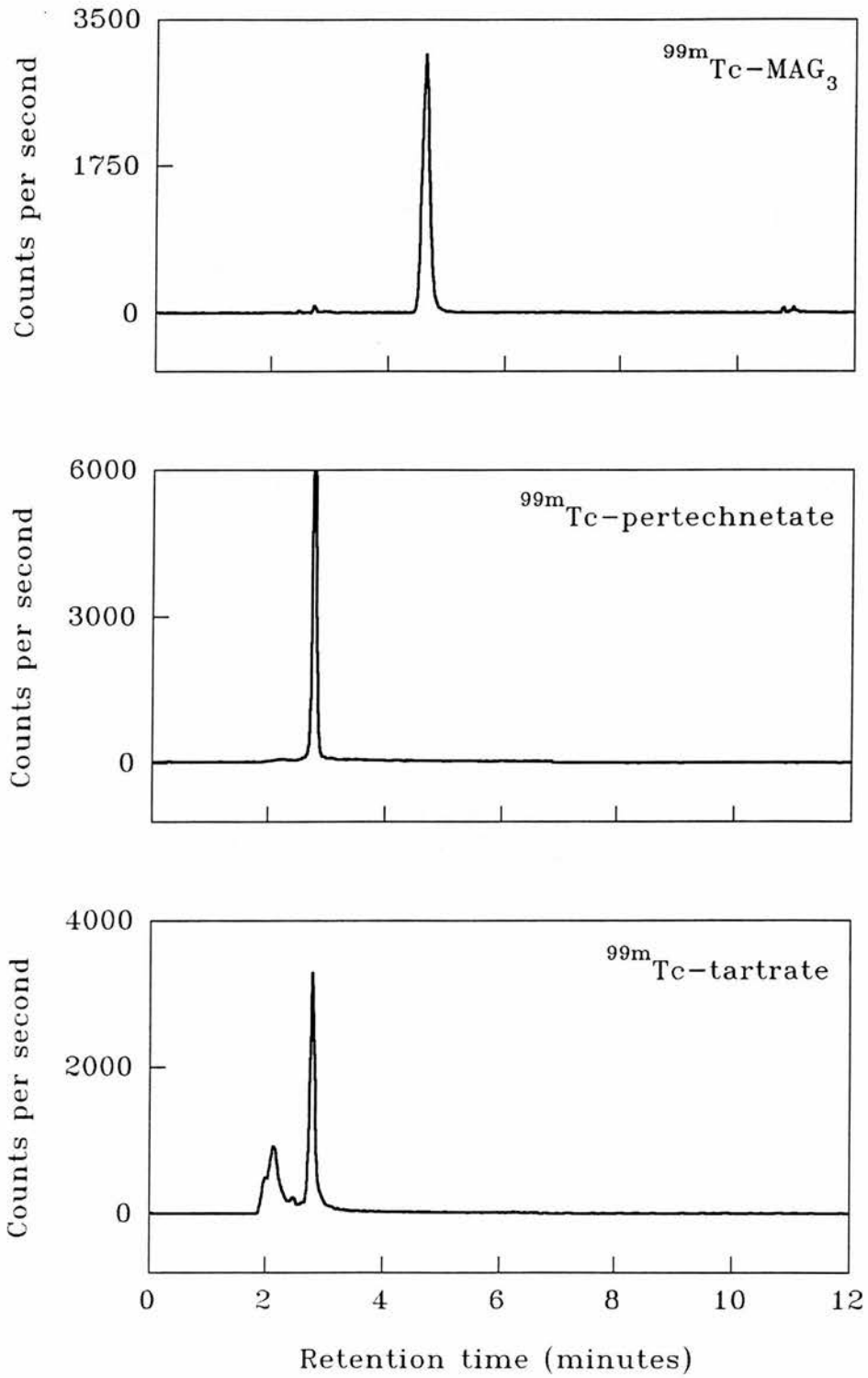

Figure 4.4 *High performance liquid chromatograms of ^{99m}Tc-MAG$_3$, ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate.*

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

Having demonstrated their ability to separate ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate from ^{99m}Tc-MAG$_3$, I decided to compare the suitability of the three techniques for determination of the radiochemical purity of ^{99m}Tc-MAG$_3$. Twelve preparations of ^{99m}Tc-MAG$_3$ with radiochemical purities of between 10% and 100% were analysed by each technique.

To achieve a wide range of radiochemical purities, two methods of obtaining ^{99m}Tc-MAG$_3$ that contained radiochemical impurities were used. The first involved taking a sample of the ^{99m}Tc/MAG$_3$ mixture before the kit was placed in the boiling water-bath. Without the high temperature of the water-bath to cleave the benzoyl group from the betiatide molecule, the exchange of ^{99m}Tc from tartrate to MAG$_3$ proceeds slowly. This mixture would be expected to contain ^{99m}Tc-tartrate and some ^{99m}Tc-MAG$_3$ complex that had formed at room temperature. The second technique was to add Sodium Pertechnetate [^{99m}Tc] Injection to ^{99m}Tc-MAG$_3$ to create a ^{99m}Tc-pertechnetate impurity. The results from HPLC are compared with those from thin-layer and paper in Figures 4.5 and 4.6 respectively.

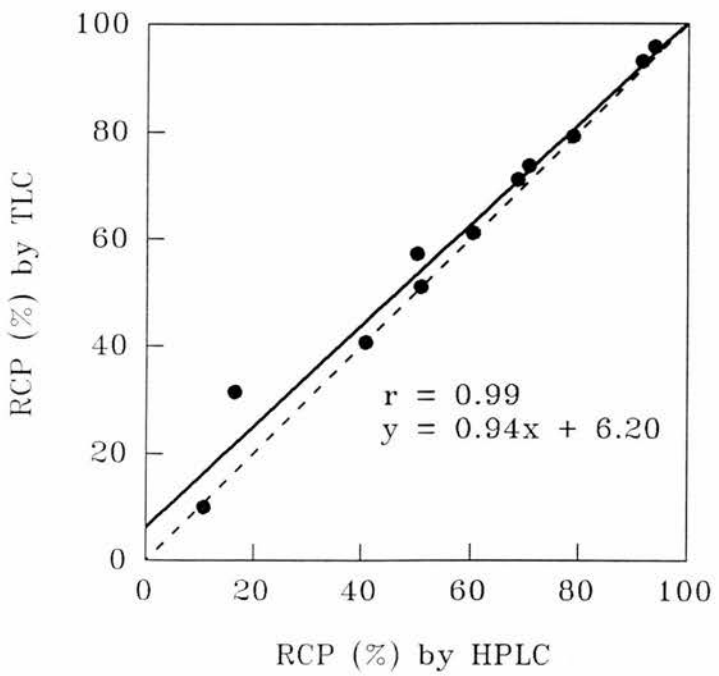

Figure 4.5 *Comparison of the radiochemical purity (RCP) of ^{99m}Tc-MAG$_3$ as measured by HPLC and thin-layer chromatography (TLC).*

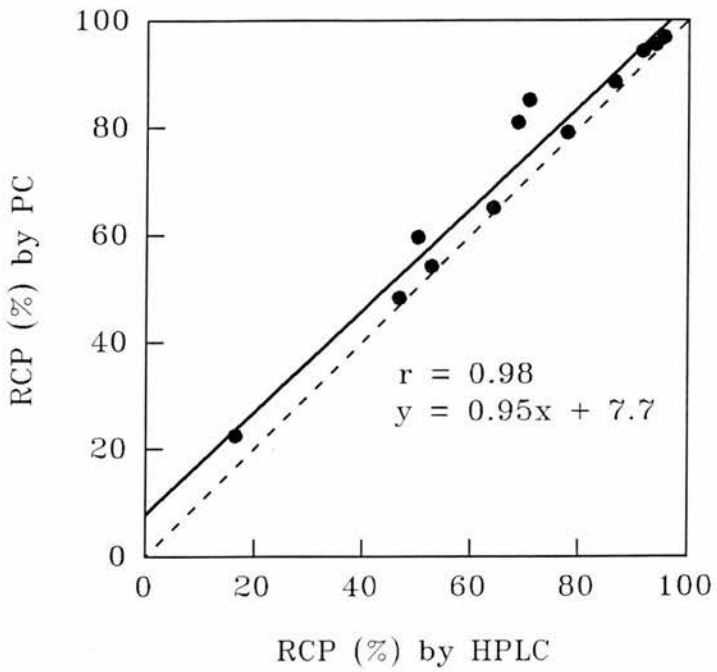

Figure 4.6 *Comparison of the radiochemical purity (RCP) of ^{99m}Tc-MAG$_3$ as measured by HPLC and paper chromatography (PC).*

Thin-layer and paper chromatography gave consistently higher results than HPLC. This might be explained by some of the ^{99m}Tc-MAG$_3$ complex being retained on the HPLC column while all the impurities in the radiopharmaceutical are completely eluted. This would have the effect of depressing the radiochemical purity result. However, the measurement of recovery from the column gave a result of 100.1 $\pm$1.4% which demonstrates that this explanation is not valid. A more likely reason for the differences is that with the thin-layer and paper chromatography techniques one or more impurities have the same chromatographic behaviour as ^{99m}Tc-MAG$_3$.

To investigate this possibility, ^{99m}Tc-MAG$_3$ was analysed by HPLC and the column eluate was collected in three fractions. The first fraction was the eluate that appeared before the ^{99m}Tc-MAG$_3$ peak (see Figure 4.9). This fraction contained the hydrophilic impurities including ^{99m}Tc-pertechnetate and ^{99m}Tc-tartrate. The second fraction was the eluate that contained the main peak of the chromatogram. This represented pure ^{99m}Tc-MAG$_3$. The third fraction contained the more lipophilic impurities that were eluted after the change of mobile phase. The three fractions were then analysed by the thin-layer and paper chromatography techniques. As a result of only 20 μl of ^{99m}Tc-MAG$_3$ being injected onto

the column, the radioactive concentrations of the samples of column eluate were extremely low. The activities that were applied to the paper and thin-layer plates were therefore correspondingly low making scanning of the plates inappropriate. The plates were therefore cut into 1 cm transverse sections which were then counted in an automatic gamma counter (Model 1282 Compugamma, LKB Wallac). The chromatograms were then created from the count-rates in the sections. The results are shown in Figures 4.7 and 4.8. With both techniques, the impurity that is eluted after the main peak on HPLC exhibits a similar chromatographic behaviour to ^{99m}Tc-MAG$_3$. On paper chromatography, one or more of the impurities that are eluted before the main peak on HPLC exhibit a similar chromatographic behaviour to ^{99m}Tc-MAG$_3$. In the presence of these impurities it would therefore be anticipated that paper and thin-layer chromatography would give higher values of radiochemical purity than HPLC and that paper chromatography would give higher results than thin-layer.

Comparison of the techniques across a range of radiochemical purities from 10% to 100% is perhaps inappropriate. It is likely that any one of the techniques will adequately demonstrate a radiochemical purity of less than 90%. Below this value, the accuracy of the result is not critical since the poor quality of the radiopharmaceutical is obvious. It is therefore more relevant to compare the techniques using ^{99m}Tc-MAG$_3$ with a radiochemical purity of between 90% and 100%. Ten preparations of ^{99m}Tc-MAG$_3$ with radiochemical purities in this range were therefore analysed by each of the three techniques. The mean results $\pm$ standard deviation were: HPLC - 94.6 $\pm$ 1.2%, thin-layer chromatography - 95.7 $\pm$ 1.3% and paper chromatography - 96.2 $\pm$ 1.2%. As in the previous experiment, thin-layer and paper gave higher results than HPLC. On analysis with a paired t-test, the differences were significant ($P<0.05$). Although statistical significance was found, the three results are reasonably similar. However, this similarity would not exist in the presence of higher levels of impurity. From the previous experiment it was concluded that the impurity which elutes after the main peak on HPLC is primarily responsible for the difference in results. The results are reasonably similar because this impurity is small (approximately 2%). In the presence of a higher concentration of the impurity HPLC would measure a much lower radiochemical purity than paper or thin-layer.

Of the three techniques, HPLC demonstrates the presence of the greatest number of impurities, gives a main peak that is well separated from the other peaks in the chromatogram and gives 100% recovery from the column. I therefore concluded that HPLC is a satisfactory technique for determination of the radiochemical purity of ^{99m}Tc-MAG$_3$.

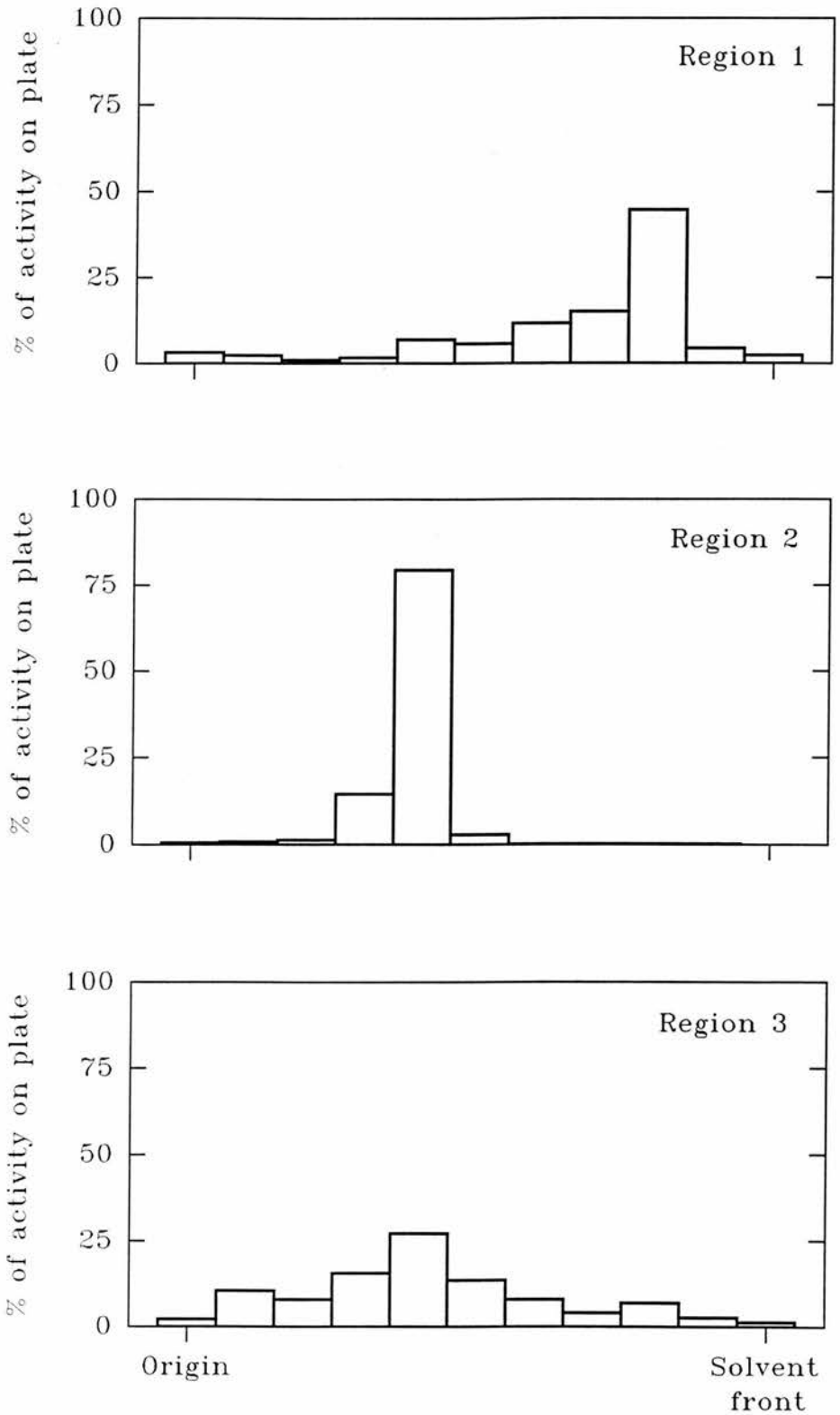

Figure 4.7 *Thin-layer chromatograms of eluate fractions from HPLC analysis.*

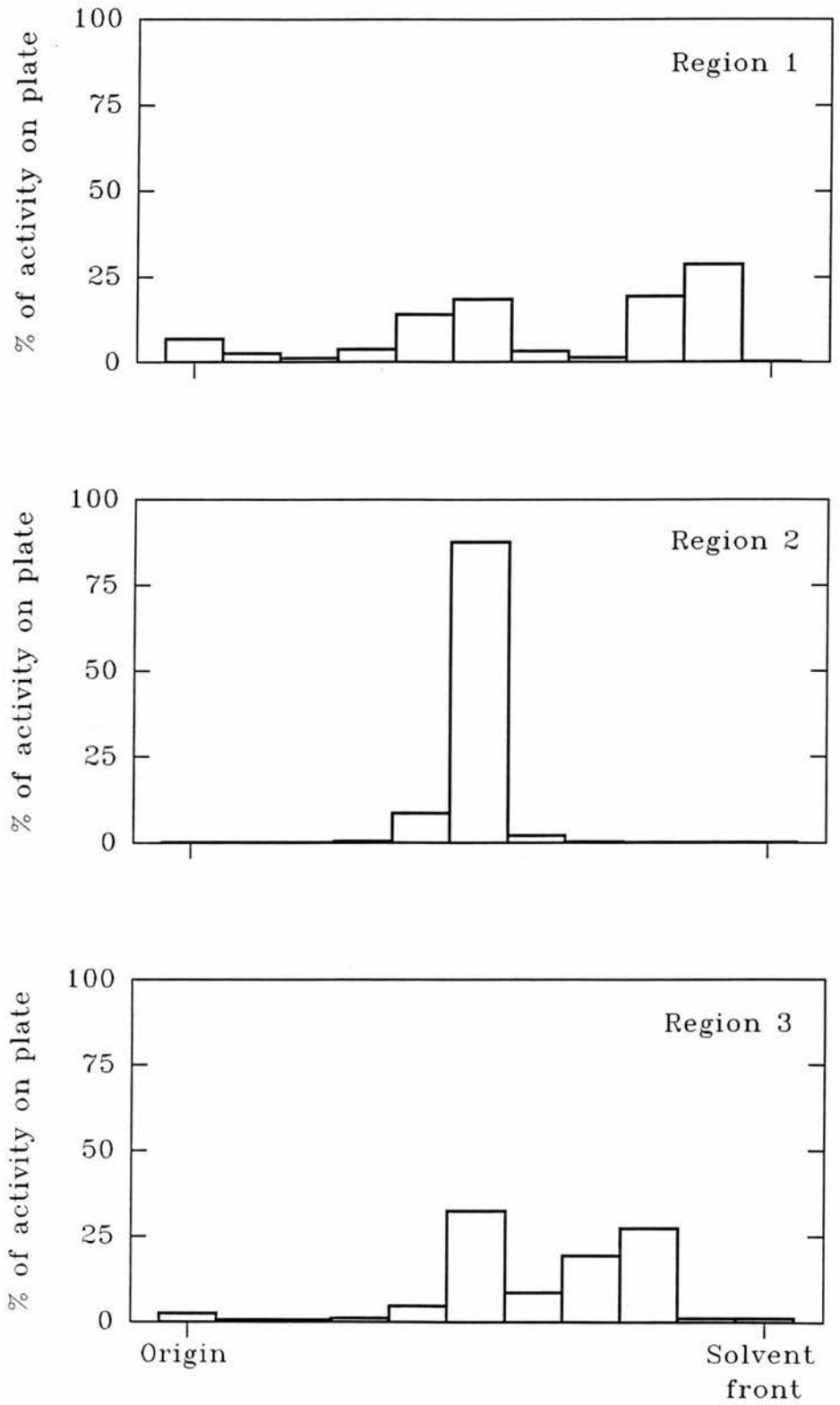

Figure 4.8 *Paper chromatograms of eluate fractions from HPLC analysis.*

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

The next stage of my investigation was to use the HPLC technique to study the radiochemical purity of ^{99m}Tc-MAG$_3$ prepared under a variety of conditions that might arise in routine radiopharmacy practice.

4.2 Radiochemical purity and stability of ^{99m}Tc-MAG$_3$ prepared at 400 MBq/4 ml

When the MAG$_3$ kit was first introduced, Mallinckrodt recommended that the ^{99m}Tc-MAG$_3$ should be administered to the patient within one hour of preparation. The reason given for this short expiry was the potential for the formation of radiochemical impurities in the ^{99m}Tc-MAG$_3$ with time. A one hour expiry was recognised as a severe drawback to the routine use of this radiopharmaceutical since nuclear medicine departments can be more than one hour's travelling time from the radiopharmacy. Even within an institution, an expiry time of one hour creates logistical problems on the use of a product and these may be sufficiently restrictive to preclude the routine use of the product. The next part of this work was therefore undertaken to investigate the validity of the one hour expiry time. Preparation of the ^{99m}Tc-MAG$_3$ was performed strictly according to the instructions supplied by the manufacturer of the MAG$_3$ kit. In view of this strict adherence to the manufacturer's instructions, I assumed that the quality of the resulting ^{99m}Tc-MAG$_3$ would represent the standard against which the results of any subsequent modifications to the preparative procedure could be judged.

4.2.1 Preparation of ^{99m}Tc-MAG$_3$

The ^{99m}Tc generator was eluted to give an eluate with a radioactive concentration of $>$400 MBq/ml. A volume of the resulting Sodium Pertechnetate [^{99m}Tc] Injection containing 400 MBq was diluted to 4 ml with Sodium Chloride Injection B.P. This solution was injected into a MAG$_3$ kit. The vial was heated, cooled and stored as described earlier. Five kits from each of three lots of MAG$_3$ kits were reconstituted according to this technique. The radiochemical purity of the ^{99m}Tc-MAG$_3$ in each vial was measured immediately after preparation, one hour after preparation, i.e. at the expiry time recommended by Mallinckrodt, and six hours after preparation, i.e. at a more typical expiry time for a ^{99m}Tc radiopharmaceutical.

4.2.2 Measurement of radiochemical purity

The radiochemical purity of the ^{99m}Tc-MAG$_3$ was measured by HPLC using the technique evaluated in the previous work. The Perkin-Elmer pump fitted with the Rheodyne 7120 injection valve and the MS310-based radiation detector with the detection cell in configuration C (Figure 2.1) were used for these analyses. As the Perkin-Elmer pump was capable of only isocratic elution, the column was eluted at 1 ml/minute with the ethanol/0.01 M phosphate buffer pH6 (5:95) mobile phase for 10 minutes then the methanol/water (90:10) mobile phase for 10 minutes. The recovery from the column was measured according to the method described in Chapter 3.4. The technique described in Chapter 3.5 was used to calculate the counts in each peak as a percentage of the total counts in the chromatogram.

To demonstrate the presence of impurities, an expanded version of the chromatogram is shown in Figure 4.9. The chromatogram reveals the existence of four hydrophilic impurities (1 - 4) that elute before the main ^{99m}Tc-MAG$_3$ peak (5) and one or more lipophilic impurities that elute in a peak (6) after the change of mobile phase. Peak 3 was shown to have the same retention time as ^{99m}Tc-pertechnetate.

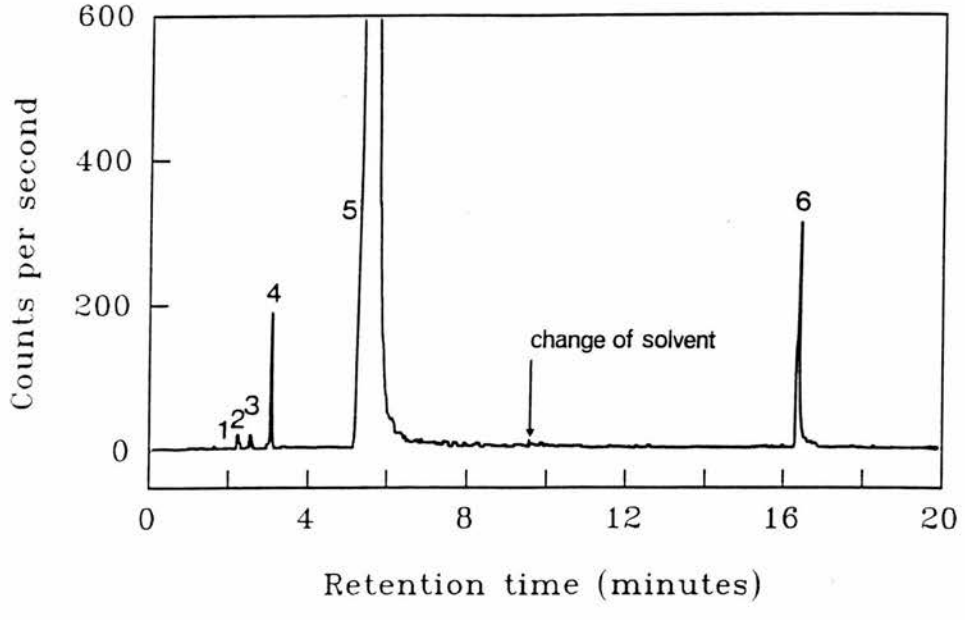

Figure 4.9 *High performance liquid chromatogram of ^{99m}Tc-MAG$_3$.*

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

Table 4.1 contains the results of the stability study performed on the three lots of MAG_3 kits. Of the impurity peaks, the only one which changes over the 6 hours is peak 4 which approximately doubles in each case. When analysed using a paired Student's t-test, this change was found to be significant ($p < 0.05$). While this increase is statistically significant, it is improbable that such a small change could be clinically significant. The increase in peak 4 is accompanied by a corresponding decrease in the percentage of total activity in the main ^{99m}Tc-MAG_3 peak (5). The other impurity peaks remain constant throughout the 6 hours. The recoveries from the column are also included in Table 4.1. The typical recovery of approximately 100% demonstrates that no radiolabelled species were retained in the column and thereby eluded detection.

Contrary to the recommended expiry time of one hour, this study has shown that ^{99m}Tc-MAG_3 prepared according to the manufacturer's instructions appears to be stable for at least 6 hours. Coveney and Robbins (1987) have also demonstrated this longer stability although their work was performed on the MAG_3 kits supplied to the North American market. These kits contain 1 mg betiatide, 40 mg sodium tartrate dihydrate, 20 mg lactose monohydrate and 200 μg stannous chloride dihydrate. This is a different formula to the kits available in Europe and the higher concentration of stannous ion could be responsible for enhanced stability. My unsuccessful attempts (unpublished data) to reproduce the cold fractionation technique of Thorson et al. (1992) in which the North American kit was used and a similar observation by Maltby (personal communication) tend to suggest that the American formula results in a more stable radiopharmaceutical.

Table 4.1 Results of the analysis of ^{99m}Tc-MAG$_3$ prepared at 400 MBq/4 ml.

Lot no. of MAG$_3$ kits	*Time after preparation (hours)*	*% of the recovered activity in each peak*						*% recovery from column*
		1	*2*	*3*	*4*	*5*	*6*	
8056	0	0.2 ± 0.2	0.3 ± 0.1	0.6 ± 0.5	0.8 ± 0.1	95.2 ± 0.7	2.9 ± 0.8	100.0 ± 3.2
	1	0.3 ± 0.3	0.3 ± 0.2	0.5 ± 0.4	1.2 ± 0.1	94.4 ± 1.2	3.3 ± 0.9	96.3 ± 3.4
	6	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.6	1.6 ± 0.2	94.2 ± 0.7	2.7 ± 0.7	99.0 ± 3.8
8068	0	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.4	0.5 ± 0.1	95.9 ± 0.7	2.5 ± 0.4	100.1 ± 0.9
	1	0.2 ± 0.1	0.3 ± 0.1	0.7 ± 0.3	0.7 ± 0.1	95.7 ± 0.6	2.4 ± 0.3	100.5 ± 0.2
	6	0.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.4	1.1 ± 0.1	95.2 ± 0.7	2.3 ± 0.2	100.4 ± 0.1
8073	0	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	96.5 ± 0.3	2.2 ± 0.2	100.7 ± 1.1
	1	0.2 ± 0.1	0.2 ± 0.0	0.5 ± 0.2	0.7 ± 0.1	96.4 ± 0.2	2.0 ± 0.1	100.8 ± 1.7
	6	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	1.2 ± 0.2	96.0 ± 0.4	1.8 ± 0.3	100.1 ± 4.7

Each value is the mean ± standard deviation of 5 results.

4.2.3 Clinical evaluation

Although the stability study has demonstrated satisfactory radiochemical purity of ^{99m}Tc-MAG$_3$ up to six hours after preparation, it is possible that the HPLC technique is not sufficiently sensitive to reveal the reason for Mallinckrodt assigning a one hour expiry to this radiopharmaceutical. The MAG$_3$ package insert contains the statement "Small amounts (1-2%) of ^{99m}Tc-labelled impurities will be formed during the labelling process. As these impurities are accumulated in the liver and gall bladder they may influence the late phase (after 30 minutes) of a dynamic renal study". The lipophilic impurities are the most likely to undergo hepatobiliary uptake and as Mallinckrodt suggest, these have been detected in the radiopharmaceutical and constitute a few percent of the total activity (peak 6). However, the proportion in this peak did not change over a period of six hours. The following clinical study to measure uptake of ^{99m}Tc into the liver and gall bladder was therefore undertaken as a means of confirming the stability of ^{99m}Tc-MAG$_3$.

Twenty patients attending for routine diuretic renography were investigated. Each patient was injected intravenously with 100 MBq of ^{99m}Tc-MAG$_3$. Ten of the patients were injected within 1 hour of preparation of the ^{99m}Tc-MAG$_3$ while the other ten were injected between 5 and 6 hours after preparation. The routine diagnostic imaging study was performed, taking 30 minutes. The gamma-camera (Digicamera, Scintronix) was then positioned to acquire anterior and right lateral static images of the abdomen. The images were recorded in the gamma-camera system's computer (Model MCS560, Technicare) to assess activity in the liver and gall bladder. Typical images are shown in Figures 4.10 and 4.11.

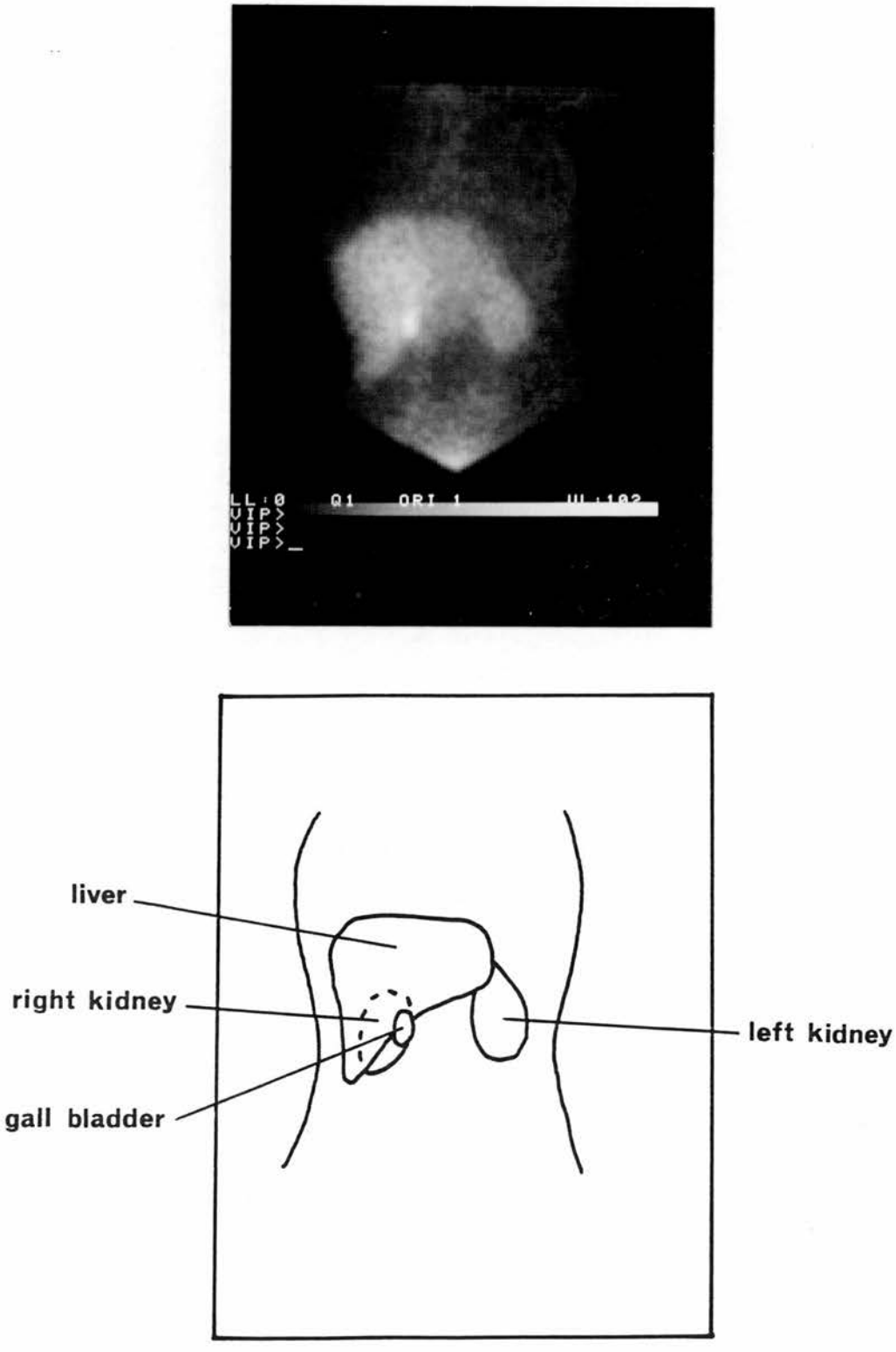

Figure 4.10 *Anterior gamma-camera image (top) and diagrammatic illustration (bottom) of residual ^{99m}Tc in the abdomen 30 minutes after the administration of ^{99m}Tc-MAG$_3$.*

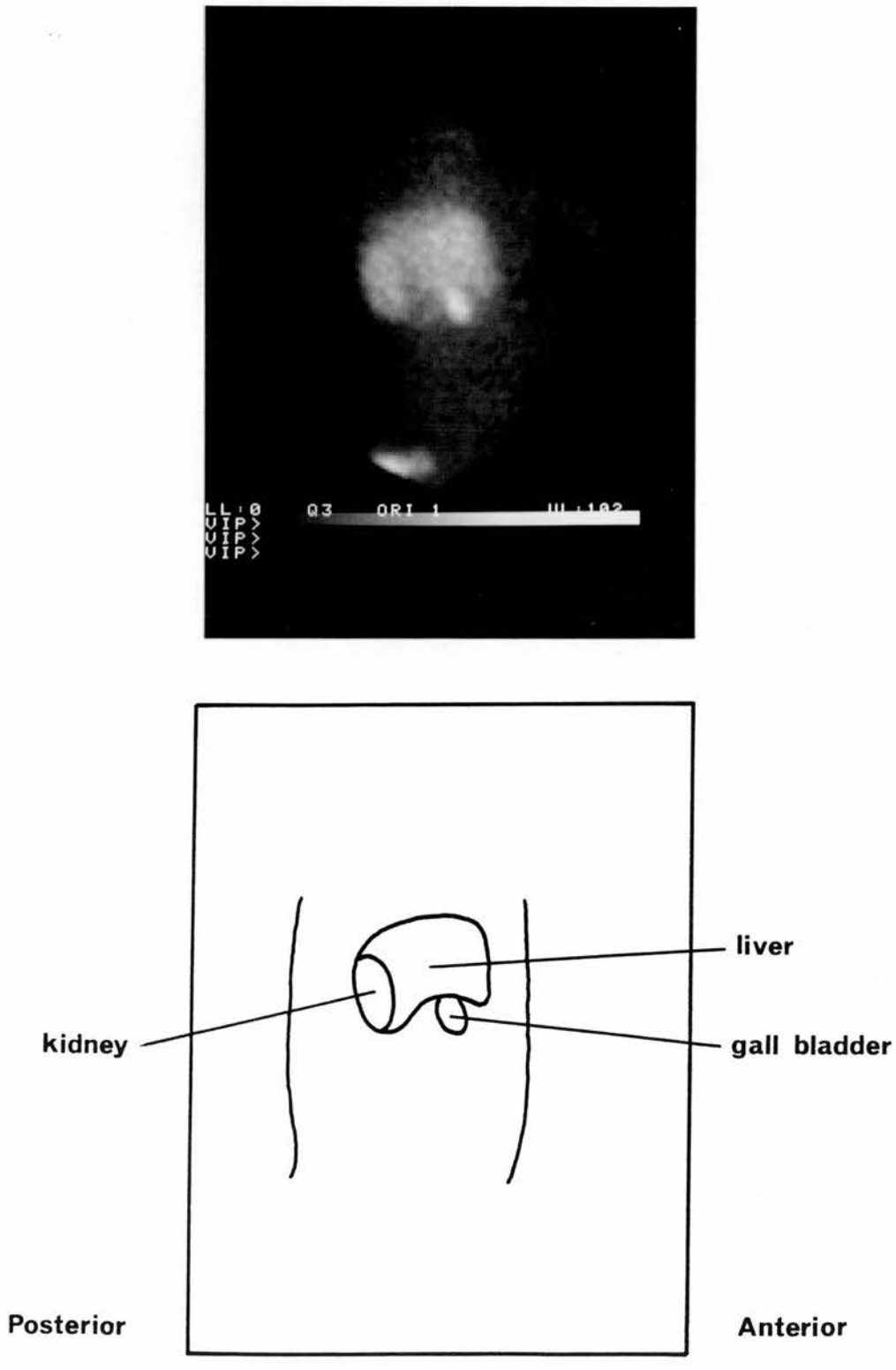

Figure 4.11 *Right lateral gamma-camera image (top) and diagrammatic illustration (bottom) of residual ^{99m}Tc in the abdomen 30 minutes after the administration of ^{99m}Tc-MAG$_3$.*

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

From the anterior image, the depth of the gall bladder in the right lateral projection was measured. On the right lateral image, four regions of interest (ROI) were created around a background area, the gall bladder, the parts of the liver that did not overlap gall bladder or residual renal activity and the whole liver. The counts and number of pixels in each of the first three ROIs, and the number of pixels in the whole liver ROI were obtained. The counts in the gall bladder and liver ROIs were corrected for background. The activity in the gall bladder was then calculated by comparing the counts in the gall bladder ROI to the counts from a ^{99m}Tc source of known activity positioned at a distance from the face of the gamma-camera equal to the depth of the gall bladder. Knowing the activity of ^{99m}Tc-MAG$_3$ that had been injected into the patient, the percentage of injected ^{99m}Tc in the gall bladder at the end of the study was calculated. The percentage of injected ^{99m}Tc in the liver was measured in the same way except that to estimate the counts in the whole liver, it was necessary to multiply the counts in the part liver ROI by the ratio of the pixels in the whole liver ROI to the part liver ROI.

To ensure that the results of this study were not influenced by disparities in the renal function of the patients in the two groups, a venous blood sample was taken from each patient for measurement of plasma creatinine. Urine was collected from six patients and analysed by HPLC to determine the form in which the ^{99m}Tc is excreted.

Table 4.2 contains the incidence of visualization of the gall bladder and the percentages of administered activity in gall bladder and liver 30 minutes after administration of ^{99m}Tc-MAG$_3$. When analysed using Wilcoxon's nonparametric test for unpaired comparison, gall bladder activity, liver activity and plasma creatinine in the <1 hour group were not found to differ significantly from those in the 5-6 hour group ($p>0.05$). Only one patient in each group had abnormal renal function as indicated by a plasma creatinine level of greater than 150 μmol/litre. It is unlikely, therefore, that the comparison was influenced by disparities in renal function between the two groups.

Table 4.3 contains the HPLC profile of the ^{99m}Tc in the urine of six patients along with the profile of a typical preparation of ^{99m}Tc-MAG$_3$ for comparison. The similarity of the two profiles suggests that the ^{99m}Tc-MAG$_3$ is excreted unchanged. The results also demonstrate that all the impurities in ^{99m}Tc-MAG$_3$ are excreted by the kidneys. However, the concentrations of impurities are lower in the urine than in the original injection showing that they are excreted less efficiently the ^{99m}Tc-MAG$_3$.

Table 4.2 Results of the clinical study performed with ^{99m}Tc-MAG$_3$ prepared at 400 MBq/4 ml.

		Time between preparation and injection	
		< 1 hour	*5 - 6 hours*
No. of images showing gall bladder		5	5
% of injected ^{99m}Tc in gall bladder	median	0.1	0.1
	(range)	(0.0 - 0.3)	(0.0 - 0.5)
% of injected ^{99m}Tc in liver	median	4.8	4.4
	(range)	(3.1 - 12.9)	(2.0 - 8.9)
Plasma creatinine (μmol/l)	median	90	106
	(range)	(66 - 529)	(79 - 230)
No. of patients with plasma creatinine > 150 μmol/l		1	1

Table 4.3 Results of the chromatographic analysis of urine compared to a typical preparation of Tc99m-MAG$_3$

	% of the recovered activity in each peak						*% recovery from*
Sample	*1*	*2*	*3*	*4*	*5*	*6*	*column*
Urine (n = 6)	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.2	0.4 ± 0.1	97.8 ± 0.6	1.3 ± 0.3	101.0 ± 2.0
Lot 8068 at 1 hour	0.2 ± 0.1	0.3 ± 0.1	0.7 ± 0.3	0.7 ± 0.1	95.7 ± 0.6	2.4 ± 0.3	100.5 ± 0.2

4. Radiochemical Purity of ^{99m}Tc-MAG$_3$ Injection

The results of this clinical study confirm the findings of the HPLC analysis in that the radiochemical purity of ^{99m}Tc-MAG$_3$ is stable over a period of six hours.

4.3 Radiochemical purity and stability of ^{99m}Tc-MAG$_3$ prepared at 1 GBq/4 ml

During the clinical study described in 4.2.3 it became apparent that less than 100 MBq of ^{99m}Tc-MAG$_3$ would be sufficient for the performance of a satisfactory study. In this next phase of my work, I decided to prepare the ^{99m}Tc-MAG$_3$ in such a way as to accommodate an administered activity of 50 MBq. Also, ^{99m}Tc-MAG$_3$ prepared at a radioactive concentration of 400 MBq/4 ml does not lend itself to routine practice in radiopharmacy where it is necessary, in the interests of economy, to obtain several patient doses from each MAG$_3$ kit. To facilitate an administered activity of 50 MBq per patient, I therefore decided that it would be appropriate to prepare single dose vials containing 175 MBq/2.5 ml. This activity would allow studies to be performed up to 6 hours after preparation of the ^{99m}Tc-MAG$_3$.

4.3.1 Preparation of ^{99m}Tc-MAG$_3$

^{99m}Tc-MAG$_3$ was prepared in an identical manner to that described in 4.2.1 except that the ^{99m}Tc generator was eluted in a volume that gave Sodium Pertechnetate [^{99m}Tc] Injection with a radioactive concentration of >1 GBq/ml. The kit was reconstituted with 1 GBq of this Sodium Pertechnetate [^{99m}Tc] Injection that had been diluted to 4 ml. This is the maximum activity recommended by Mallinckrodt for reconstitution of the MAG$_3$ kit. After the 10 minute cooling stage in the beaker of water at room temperature, individual patient doses were dispensed by withdrawing a 0.7 ml aliquot from the kit, diluting it to 2.5 ml with Sodium Chloride Injection and injecting the solution into a sterile 10 ml vial containing a nitrogen atmosphere (Product Code N46, Amersham). The vial was then stored at room temperature. ^{99m}Tc-MAG$_3$ from five kits was prepared according to this technique. As before, radiochemical purity was measured by HPLC at 0, 1 and 6 hours after preparation.

4.3.2 Measurement of radiochemical purity

Analysis by HPLC was carried out as described earlier except that the radiation detector was

used in configuration B to accommodate the higher radioactive concentration of the samples. Table 4.4 contains the results. These are similar to the results obtained with the "gold standard" ^{99m}Tc-MAG$_3$ prepared at 400 MBq/4 ml. Two aspects of the results do however differ. Peak 3, which has the same retention time as ^{99m}Tc-pertechnetate, contains a higher percentage of the total activity in the more dilute preparation. This could be caused by the greater demand placed on the labelling reaction by more Tc being used. Alternatively, the introduction of oxygen that would inevitably be present in the Sodium Chloride Injection used to dilute the labelled product, could induce oxidation of the ^{99m}Tc-MAG$_3$ and result in the release of ^{99m}Tc-pertechnetate. The second difference is that peak 4 does not double over the six hours of the study. This suggests that dilution has a stabilizing effect on ^{99m}Tc-MAG$_3$. However, the magnitude of these effects is so small that they are unlikely to influence the clinical efficacy of the radiopharmaceutical. As before, the radiochemical purity of the ^{99m}Tc-MAG$_3$ was greater than 95% and the recoveries from the HPLC column were satisfactorily high.

Table 4.4 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 1 GBq/4 ml followed by subdivision and dilution.

Lot no. of MAG$_3$ kits	*Time after preparation (hours)*	*% of the recovered activity in each peak*						*% recovery from column*
		1	*2*	*3*	*4*	*5*	*6*	
8073	0	0.1 ± 0.1	0.3 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	96.1 ± 0.3	2.3 ± 0.2	97.8 ± 1.5
	1	0.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.4	0.5 ± 0.1	96.0 ± 0.4	2.2 ± 0.3	97.9 ± 2.2
	6	0.1 ± 0.1	0.3 ± 0.1	1.1 ± 0.2	0.4 ± 0.1	95.7 ± 0.3	2.4 ± 0.1	98.0 ± 2.6

Each value is the mean ± standard deviation of 5 results.

4.3.3 Clinical evaluation

The in-vivo behaviour of the ^{99m}Tc-MAG$_3$ was evaluated as before in a second group of 20 patients. Table 4.5 contains the results which are in agreement with the results from the first study.

Table 4.5 Results of the clinical study performed with ^{99m}Tc-MAG$_3$ prepared at 1 GBq/4 ml followed by subdivision and dilution.

		Time between preparation and injection	
		< 1 hour	*5 - 6 hours*
No. of images showing gall bladder		3	4
% of injected ^{99m}Tc in gall bladder	median	0.0	0.0
	(range)	(0.0 - 0.1)	(0.0 - 0.4)
% of injected ^{99m}Tc in liver	median	4.7	4.0
	(range)	(1.4 - 12.3)	(2.3 - 12.4)
Plasma creatinine (μmol/l)	median	97	95
	(range)	(63 - 290)	(63 - 324)
No. of patients with plasma creatinine > 150 μmol/l		2	2

From these results I concluded that ^{99m}Tc-MAG$_3$ is stable for six hours when prepared according to the methods used in this investigation. Arising from this work, ^{99m}Tc-MAG$_3$ prepared in this department is now given an expiry time of six hours after preparation.

4.4 Effect of other factors on radiochemical purity and stability

The ^{99m}Tc-MAG$_3$ injections used so far in this investigation have been prepared using techniques that do not differ markedly from that recommended by Mallinckrodt. However, while the manufacturer's instructions specify a number of conditions for the labelling procedure, they do not cover all variables that might affect the quality of the labelled product. The remainder of this work was therefore undertaken to determine the effect of various parameters on the quality of ^{99m}Tc-MAG$_3$ and to identify the optimal preparative conditions for high radiochemical purity and stability.

4.4.1 Volume of ^{99m}Tc generator eluate

The Mallinckrodt instructions specify that the MAG$_3$ kit should be reconstituted with 4 ml Sodium Pertechnetate [^{99m}Tc] Injection, less than 1.0 ml of which is ^{99m}Tc generator eluate. It could be assumed, therefore, that if more than 1.0 ml of generator eluate is used, a product of inferior quality is produced. Conversely, it might be that the smaller the volume of generator eluate used, the superior the product obtained. To test this hypothesis, ^{99m}Tc-MAG$_3$ was prepared using the volumes of eluate at either end of the recommended range, i.e. 0.1 and 1.0 ml, to determine if using the lower volume would be a means of obtaining a product with a higher radiochemical purity.

With increasing clinical experience of ^{99m}Tc-MAG$_3$, the activities of 100 and 50 MBq that had been administered in the clinical studies were found to be higher than were necessary to perform satisfactory investigations. Therefore, while the 1 GBq used to reconstitute the kit in 4.3 would provide more than enough ^{99m}Tc-MAG$_3$ to meet any demands placed on the radiopharmacy service, it would undoubtedly result in the unnecessary handling of ^{99m}Tc and the associated increase in exposure of the radiopharmacy staff to radiation. The use of a lower activity would have the benefit of reducing the radiation dose to staff and patients. For this series of experiments, I therefore decided to prepare the ^{99m}Tc-MAG$_3$ at a radioactive concentration of 500 MBq/4 ml. Preparation was carried out as described in 4.2.1. As before, the final product was stored at room temperature. Radiochemical purity was measured by HPLC immediately after preparation and 6 hours later. I decided to omit the measurement at 1 hour after preparation since it had not revealed any short-term instability.

The results are contained in Table 4.6 and show that the volume of eluate had no effect on the radiochemical purity. As observed previously the content of peak 4 approximately doubled over the six hours of the study.

Table 4.6 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 500 MBq/4 ml using 0.1 ml and 1.0 ml of generator eluate.

Conditions for preparation	*Time after preparation (hours)*	*% of the recovered activity in each peak*						*% recovery from column*
		1	*2*	*3*	*4*	*5*	*6*	
0.1 ml eluate	0	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	96.4 ± 0.4	2.4 ± 0.5	99.0 ± 2.3
	6	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	1.3 ± 0.3	96.2 ± 0.2	1.8 ± 0.2	99.6 ± 2.3
1.0 ml eluate	0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	96.7 ± 0.3	2.3 ± 0.2	101.5 ± 1.2
	6	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.2	1.2 ± 0.2	96.2 ± 0.2	1.9 ± 0.1	101.5 ± 1.8

Each value is the mean ± standard deviation of 5 results.

4.4.2 Dilution, aeration and agitation

In radiopharmacies that supply a number of nuclear medicine departments, a policy of preparing individual patient doses of radiopharmaceuticals is often adopted. Subdividing kits of ^{99m}Tc radiopharmaceuticals after preparation is therefore a common practice. This process of subdivision can be accompanied by dilution during which air can inadvertently be introduced into the vial. In certain situations, the individual patient doses can be transported some considerable distance to the nuclear medicine department. During transport, the

radiopharmaceuticals are invariably subjected to agitation. The effect of dilution, aeration and agitation of ^{99m}Tc-MAG$_3$ was therefore studied in this experiment.

^{99m}Tc-MAG$_3$ was prepared as in 4.4.1 using ^{99m}Tc generator eluate with a radioactive concentration of 500 MBq/$<$1.0 ml. A 0.7 ml aliquot of the labelled product was then transferred to a 10 ml nitrogen-filled sterile vial (Product Code N46, Amersham) and diluted to 2.5 ml with Sodium Chloride Injection. Air (10 ml) was bubbled through the solution and then the vial was placed on a rotating axle mixer (Spiramix 5, Denley) for 6 hours. The radiochemical purity of the ^{99m}Tc-MAG$_3$ was measured by HPLC immediately after preparation and 6 hours later.

The results are contained in Table 4.7 and show that dilution, aeration and agitation have little effect on radiochemical purity. As was observed in 4.3, dilution results in a slightly but not unacceptably higher level of ^{99m}Tc-pertechnetate impurity. As was also observed in 4.3, dilution appears to eliminate the doubling of peak 4 over a six hour period.

Table 4.7 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 500 MBq/4 ml using $<$1.0 ml of generator eluate followed by dilution, aeration and agitation.

Time after preparation (hours)	*% of the recovered activity in each peak*						*% recovery from column*
	1	*2*	*3*	*4*	*5*	*6*	
0	0.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.5	0.4 ± 0.1	95.9 ± 0.7	2.4 ± 0.3	101.0 ± 2.9
6	0.1 ± 0.1	0.3 ± 0.1	1.0 ± 0.5	0.5 ± 0.2	95.8 ± 0.9	2.3 ± 0.3	100.6 ± 2.4

Each value is the mean ± standard deviation of 5 results.

4.4.3 Reconstitution volume

At this point in my study, Mallinckrodt Medical issued new recommendations for the preparation of ^{99m}Tc-MAG$_3$. The total volume of Sodium Pertechnetate [^{99m}Tc] Injection to be added to the kit was increased from 4 ml to 10 ml, up to 3 ml of which could be generator eluate. Under these revised conditions, the recommended shelf-life of the labelled product was increased from 1 to 4 hours. The effect of this revised procedure was therefore investigated by preparing ^{99m}Tc-MAG$_3$ at an activity of 500 MBq as before but at a volume of 10 ml. As in the previous experiments a generator eluate volume of <1.0 ml was used since the radioactive concentration of the eluates available routinely was >500 MBq/ml. Radiochemical purity was measured as before. The results are contained in Table 4.8 and are similar to those obtained previously.

Table 4.8 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 500 MBq/10 ml.

Time after preparation (hours)	*% of the recovered activity in each peak*						*% recovery from column*
	1	*2*	*3*	*4*	*5*	*6*	
0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	96.4 ± 0.5	2.8 ± 0.4	99.0 ± 3.0
6	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.0	0.9 ± 0.1	96.2 ± 0.5	2.5 ± 0.2	99.9 ± 3.1

Each value is the mean ± standard deviation of 5 results.

4.4.4 Aeration before boiling

In an earlier experiment (4.4.2), the effect of bubbling air through the labelled product was investigated. I therefore decided also to study the effect of introducing air before the boiling step of the labelling procedure since it is conceivable that this could occur during the routine preparation of ^{99m}Tc-MAG$_3$. The ^{99m}Tc-MAG$_3$ was prepared as in the previous experiment but 10 ml of air was bubbled through the solution before the vial was placed in the boiling water bath. Radiochemical purity was measured as before. The results are contained in Table 4.9 and are similar to those obtained previously. The introduction of air before the labelling reaction is initiated does not therefore affect radiochemical purity.

Table 4.10 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 500 MBq/10 ml and with air bubbled through the reaction mixture before boiling.

Time after preparation (hours)	*% of the recovered activity in each peak*						*% recovery from column*
	1	*2*	*3*	*4*	*5*	*6*	
0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	96.8 ± 0.5	2.5 ± 0.4	99.8 ± 3.5
6	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.9 ± 0.1	96.3 ± 0.4	2.5 ± 0.3	99.3 ± 2.1

Each value is the mean ± standard deviation of 5 results.

4.4.5 Concentration of ^{99}Tc in generator eluate

On certain days of the week, principally Mondays, it is not possible to avoid preparing ^{99m}Tc radiopharmaceuticals with Sodium Pertechnetate [^{99m}Tc] Injection that has been obtained from a generator that has remained uneluted for a few days. Under these conditions, the level of Tc99 in the eluate is high. It is theoretically possible that the use of this unusually high concentration of Tc can be deleterious to the quality of radiopharmaceuticals. The effect of Tc99 was therefore investigated by preparing ^{99m}Tc-MAG$_3$ at a radioactive concentration of 500 MBq/10 ml as before but using ^{99m}Tc from the first eluate from a new generator. From the time between the last separation of molybdenum and Tc performed by the generator manufacturer and the time of the first elution in the radiopharmacy, it is possible to calculate that the ratio of ^{99}Tc:^{99m}Tc in the eluate was 16 (Bauer and Pabst 1982). Radiochemical purity was measured as before. The results are contained in Table 4.10 and show that this excess of Tc99 does not affect radiochemical purity.

Table 4.10 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 500 MBq/10 ml using a generator eluate with a ^{99}Tc/^{99m}Tc ratio of 16:1.

Time after preparation (hours)	*% of the recovered activity in each peak*						*% recovery from column*
	1	*2*	*3*	*4*	*5*	*6*	
0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	96.9 ± 0.1	2.3 ± 0.2	100.1 ± 1.3
6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.8 ± 0.1	96.7 ± 0.3	2.1 ± 0.2	99.2 ± 0.6

Each value is the mean ± standard deviation of 5 results.

4.4.6 "Wet labelling"

Preparation of ^{99m}Tc-MAG$_3$ according to the manufacturer's revised instructions involves the withdrawal of the required volume of Sodium Pertechnetate [Tc99m] Injection into a syringe, dilution of the injection to 10 ml with Sodium Chloride Injection and injection of the solution into the MAG$_3$ kit. This is a time-consuming process which exposes the fingers of the member of staff carrying out the procedure to radiation. I therefore decided to investigate the following means of minimizing the handling of the ^{99m}Tc. This procedure is a modification of the "wet labelling" technique that has been in use in this department for many years (Millar et al. 1981) and involves reconstitution of the kit with Sodium Chloride Injection before addition of the ^{99m}Tc.

The MAG$_3$ kit was first reconstituted with 9.0-9.9 ml of Sodium Chloride Injection. Sodium Pertechnetate [^{99m}Tc] Injection (500 MBq/$<$1.0 ml) was then injected into the vial to give a total volume of 10 ml. Heating, cooling and analysis of radiochemical purity were performed as before. The results are contained in Table 4.11 and show that this procedure gives ^{99m}Tc-MAG$_3$ of comparable radiochemical purity and stability to that prepared by the recommended technique. When using this modified technique, the small volume of generator eluate can be transferred quickly to the MAG$_3$ kit thus minimizing handling time and exposure of the fingers to radiation.

Table 4.11 Results of the chromatographic analysis of Tc99m-MAG$_3$ prepared at 500 MBq/4 ml by reconstitution of the kit with Sodium Chloride Injection before addition of the generator eluate.

Time after preparation (hours)	*% of the recovered activity in each peak*						*% recovery from column*
	1	*2*	*3*	*4*	*5*	*6*	
0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	97.3 ± 0.1	2.1 ± 0.1	98.6 3.3
6	0.0 ± 0.0	0.0 ± 0.1	0.2 ± 0.1	0.8 ± 0.1	96.8 ± 0.2	2.1 ± 0.1	101.9 ± 1.5

Each value is the mean ± standard deviation of 5 results.

By this stage of the study, clinical experience had shown that satisfactory clinical investigations could be performed with 10-20 MBq of ^{99m}Tc-MAG$_3$. The following experiments were therefore performed to investigate the feasibility of dispensing ten 50 MBq doses from each MAG$_3$ kit. ^{99m}Tc-MAG$_3$ was prepared as in the previous experiment. A 1.0 ml aliquot of the ^{99m}Tc-MAG$_3$ solution and 1.5 ml of Sodium Chloride Injection were injected into a sterile, nitrogen-filled, 10 ml vial. The radiochemical purities of the undiluted and diluted ^{99m}Tc-MAG$_3$ were measured by HPLC as before. This procedure was carried out five times, a kit from a different batch of MAG$_3$ being used on each occasion. The results are contained in Table 4.12 and show that this technique provides ^{99m}Tc-MAG$_3$ of satisfactorily high radiochemical purity and stability. As has been observed in previous experiments, the diluted ^{99m}Tc-MAG$_3$ does not exhibit the doubling of peak 4 that is seen with the undiluted product. This again suggests that dilution confers stability on ^{99m}Tc-MAG$_3$ in solution.

Table 4.12 Results of the comparison of undiluted and diluted ^{99m}Tc-MAG$_3$.

Conditions for preparation	*Time after preparation (hours)*	*% of the recovered activity in each peak*						*% recovery from column*
		1	*2*	*3*	*4*	*5*	*6*	
Undiluted	0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	97.5 ± 0.3	1.9 ± 0.2	96.8 ± 2.2
	6	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.8 ± 0.1	97.1 ± 0.5	1.7 ± 0.4	98.0 ± 1.2
Diluted	0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	97.5 ± 0.4	1.8 ± 0.4	98.4 ± 1.7
	6	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	97.1 ± 0.4	21. ± 0.3	100.4 ± 1.3

Each value is the mean ± standard deviation of 5 results.

4.5 Summary

In the work described in this chapter I have:

1. developed an HPLC technique for determining the radiochemical purity of ^{99m}Tc-MAG$_3$

2. demonstrated that the HPLC technique is superior to thin-layer and paper chromatographic techniques for measuring the radiochemical purity of ^{99m}Tc-MAG$_3$

3. demonstrated that the recovery of ^{99m}Tc-MAG$_3$ from the HPLC column is consistently in the order of 100%

4. used the HPLC technique to demonstrate that ^{99m}Tc-MAG$_3$, prepared under the conditions described, is stable for six hours rather than the 1 hour quoted by the manufacturer, and confirmed this finding with clinical studies in patients

5. used HPLC to demonstrate that the radiochemical purity and stability of ^{99m}Tc-MAG$_3$ is not influenced by the volume of ^{99m}Tc generator eluate used, dilution, agitation, the presence of air in the reaction vial or the use of a ^{99m}Tc generator eluate with a ^{99}Tc:^{99m}Tc ratio of 16:1

6. developed a modified method of preparing ^{99m}Tc-MAG$_3$ which should help to minimize the radiation dose to the fingers of members of the radiopharmacy staff

From these findings, it can be concluded that the HPLC technique is a satisfactory means of measuring the radiochemical purity of ^{99m}Tc-MAG$_3$.

5. Radiochemical Purity of ^{99m}Tc-DMSA Injection

The work described in this chapter was undertaken to develop an HPLC method for determining the radiochemical purity of ^{99m}Tc-Dimercaptosuccinic Acid Injection and then to use the HPLC technique in an investigation of the effects of factors that influence the preparation of this radiopharmaceutical in routine practice.

5.1 Introduction

Technetium-99m Dimercaptosuccinic Acid Injection (^{99m}Tc-DMSA) was introduced by Lin et al. (1974) as a radiopharmaceutical for imaging of the kidneys. Since then, kits for the preparation of this commonly used radiopharmaceutical have been marketed by several manufacturers.

Following intravenous injection, ^{99m}Tc-DMSA is taken up and fixed by the proximal tubules of the kidneys. Six hours after intravenous administration to a patient with normal kidneys, 20 - 35% of the injected ^{99m}Tc has become localised in each kidney and 10 - 15% has been excreted in the urine. Subsequent imaging with a gamma-camera provides good definition of the cortical outline with diseased tissue appearing on the image as areas of reduced or no activity. Kidney imaging with ^{99m}Tc-DMSA is a commonly performed investigation for the detection of conditions such as calculi, tumours, cysts, abscesses and scarring.

The uptake of the radiopharmaceutical is dependent on the presence of viable kidney tissue, therefore, in addition to providing images which demonstrate purely morphological detail, the uptake of ^{99m}Tc-DMSA can be used as an indicator of kidney function. Measurement of the relative uptake of ^{99m}Tc-DMSA into each kidney is a procedure that is used routinely to determine relative kidney function. The results obtained with this technique are not influenced by the absolute uptake of the ^{99m}Tc-DMSA, i.e. the activity of ^{99m}Tc in each kidney expressed as a percentage of injected activity, since both kidneys are affected equally by any factors, such as radiochemical purity, that might influence uptake. It has been suggested, however, that the absolute uptake of ^{99m}Tc-DMSA by a kidney can be used as a measure of absolute function. In this situation, the radiochemical purity of the ^{99m}Tc-DMSA will influence the result of the investigation. If ^{99m}Tc-DMSA with significant levels

of radiochemical impurities is used, less ^{99m}Tc will be present in the form that is concentrated by the kidneys and low uptake will be observed. A high radiochemical purity is therefore necessary if ^{99m}Tc-DMSA is to be used in this way.

Moretti et al. (1982) and Saccavini (personal communication) have reported that when sodium pertechnetate [^{99m}Tc] containing a high concentration of ^{99}Tc is used to prepare ^{99m}Tc-DMSA, a radiopharmaceutical of low radiochemical purity is produced. In the course of their work, HPLC was used for analysis of the ^{99m}Tc-DMSA but the recovery from the column was not reported. The validity of the technique is therefore open to question and the results must be treated with caution. The study demonstrated that when ^{99m}Tc-DMSA is prepared at a DMSA/Tc ratio of 10^4, a single radiolabelled species is formed and the radiopharmaceutical is concentrated by the kidney. However, when the ^{99m}Tc-DMSA is prepared at a DMSA/Tc ratio of 10^2, several radiolabelled species are formed and the radiopharmaceutical is concentrated less avidly by the kidneys. The possibility of varying amounts of Tc being used arises from the length of time for which the ^{99m}Tc generator has remained uneluted before being used to provide ^{99m}Tc for the preparation of the ^{99m}Tc-DMSA. The following calculations illustrate this situation.

The Amersham International kit for the preparation of ^{99m}Tc-DMSA contains 1 mg of DMSA. The molecular weight of DMSA is 182.22. The kit therefore contains 5.5×10^{-6} moles of DMSA. Applying Avogadro's number, this represents 3.3×10^{18} molecules of DMSA.

Amersham International recommend that the DMSA kit is reconstituted with a maximum of 1.48 GBq of ^{99m}Tc. Using equation 5.1, the number of atoms of ^{99m}Tc in 1.48 GBq is found to be 4.6×10^{13}.

$$N = \frac{t_{½}\ A}{0.693} \qquad \text{(Eq 5.1)}$$

where N = number of atoms in the source
$t_{½}$ = the half-life of the radionuclide expressed in seconds
A = the activity of the source expressed in Bq

Assuming that the 1.48 GBq of ^{99m}Tc is obtained from a generator, the previous elution of which was 24 hours earlier, equation 5.2 (Bauer & Pabst, 1982) can be used to calculate

that the ratio of ^{99}Tc to ^{99m}Tc in the eluate is 2.6.

$$\frac{^{99}TC}{^{99m}TC} = \frac{k_T - k_M}{w\,k_M} \frac{1 - e^{-k_M t}}{e^{-k_M t} - e^{-k_T t}} - 1 \qquad \text{(Eq. 5.2)}$$

where k_T = decay constant of ^{99m}Tc
k_M = decay constant of ^{99}Mo
t = time lapse
w = the probability of ^{99}Mo decaying to ^{99m}Tc

The number of atoms of ^{99}Tc present in the 1.48 GBq of ^{99m}Tc is therefore 1.2×10^{14}. The total number of atoms of Tc used to reconstitute the DMSA kit is therefore 1.7×10^{14}. The DMSA/Tc ratio in the kit is therefore 1.9×10^4 which is higher than the ratio found to be satisfactory by Moretti et al. (1982).

If the ^{99m}Tc generator remains uneluted for a five day period such as a bank holiday weekend or the interval between manufacture and first elution in the radiopharmacy, the ^{99}Tc/^{99m}Tc ratio in the eluate is 27.8. The number of atoms of Tc in 1.48 GBq of ^{99m}Tc is therefore 1.3×10^{15} giving a DMSA/Tc ratio of 2.5×10^3. After a five day period, the ratio does not fall to the level that was found to be unsatisfactory by Moretti et al. (1982).

To achieve the DMSA/Tc ratio of 10^2 that was found unsatisfactory by Moretti et al. (1982), the generator must remain uneluted for 17 days. This situation is inconceivable in routine radiopharmacy practice. A 50 GBq generator, which is a higher activity generator than is found in many radiopharmacies, would yield only 700 MBq after 17 days. This is a lower activity than is required in all but the smallest of radiopharmacies. It is also less than half of the maximum activity that Amersham recommend for reconstitution of their DMSA kit. A DMSA/Tc ratio of 10^2 is therefore of little practical interest.

The work described in this chapter was therefore carried out to:

1. determine the validity of the HPLC technique of Moretti et al. (1982)

2. develop and validate an HPLC technique for measuring the radiochemical purity of ^{99m}Tc-DMSA

3. use HPLC to determine if the radiochemical purity of ^{99m}Tc-DMSA is affected by the levels of ^{99}Tc that may be present in Sodium Pertechnetate [^{99m}Tc] Injection in routine practice

4. use HPLC to determine if the technique of "wet labelling" influences the radiochemical purity of ^{99m}Tc-DMSA

5.2 Analysis of ^{99m}Tc-DMSA on Hypersil-ODS

The purpose of the first part of this investigation was to reproduce the work of Moretti et al. (1982). The equipment used was the Philips PU4100 pump, the radiation detector in configuration A (Figure 2.1), the Accuspec system and a 100 x 5 mm Hypersil-ODS column. The chromatographic conditions were a linear gradient elution of the column at a flow-rate of 1 ml/minute. The column was equilibrated with 10 mM phosphate buffer pH3 for at least 30 minutes. Immediately after injection of the sample of ^{99m}Tc-DMSA, the elution gradient was started. This changed the mobile phase from 100% 10 mM phosphate buffer pH3 to 100% 10 mM phosphate buffer pH6 over 10 minutes.

The ^{99m}Tc-DMSA was prepared by reconstituting a DMSA kit (Product Code N107, Amersham). This kit contains 1.00 mg DMSA, 0.42 mg stannous chloride dihydrate, 0.70 mg ascorbic acid, 2.90 mg sodium chloride and 50.00 mg inositol as a freeze-dried powder in a rubber-capped vial with a nitrogen atmosphere. Reconstitution was carried out with Sodium Pertechnetate [^{99m}Tc] Injection (1.5 GBq/2 ml). This is the maximum activity recommended by Amersham for reconstitution of this kit. The previous elution of the generator from which the Sodium Pertechnetate [^{99m}Tc] Injection was obtained had been 24 hours earlier. These conditions result in a DMSA/Tc ratio of 10^4. Three hours after reconstitution of the kit, a 20 μl sample of the ^{99m}Tc-DMSA was injected onto the HPLC column. Recovery of ^{99m}Tc from the column was measured as described in Chapter 3.4. A chromatogram containing one peak with a retention time of 12.5 minutes was detected. The chromatogram is shown in Figure 5.1. Recovery from the column was only 65%. Once the chromatogram had been acquired, the column was removed from the instrument and scanned using the equipment described in Chapter 2.10 and shown in Figure 2.6. The profile of the ^{99m}Tc retained on the column is shown in Figure 5.2. All the unrecovered ^{99m}Tc was detected at the top of the column. To determine if the ^{99m}Tc had become adsorbed on the stainless steel frit at the top of the column or on the packing material, the column was placed over the gamma-counter used to determine column recoveries (Figure

2.4). The column was positioned upside-down with its top nearest to the detector and the count-rate was measured. The Shandon column system proved ideal for this procedure since the top frit is exposed when the column is removed from the instrument. The stainless steel frit was replaced and the count-rate from the column was remeasured. From these count-rates it was calculated that only 5% of the ^{99m}Tc retained at the top of the column was associated with the frit. Most of the ^{99m}Tc had therefore become bound to the column packing. The ^{99m}Tc that appeared to be adsorbed on the frit may have been adsorbed on particles of column packing that were trapped in the pores of the frit rather than on the stainless steel itself.

In their experiments, Moretti et al. (1982) used a 150 x 4.6 mm ultrasphere ODS column. The particle size of the packing material is not quoted. With this column and the chromatographic conditions described above, a single peak with a retention time of approximately 15 minutes was detected. On the assumption that the column packings in our respective experiments had the same particle size and retention properties, correction of the 15 minute retention time for the smaller column size used in my experiment gives a retention time of 11.8 minutes. Given the assumptions made, the 12.5 minute retention time observed in my experiment is in reasonable agreement.

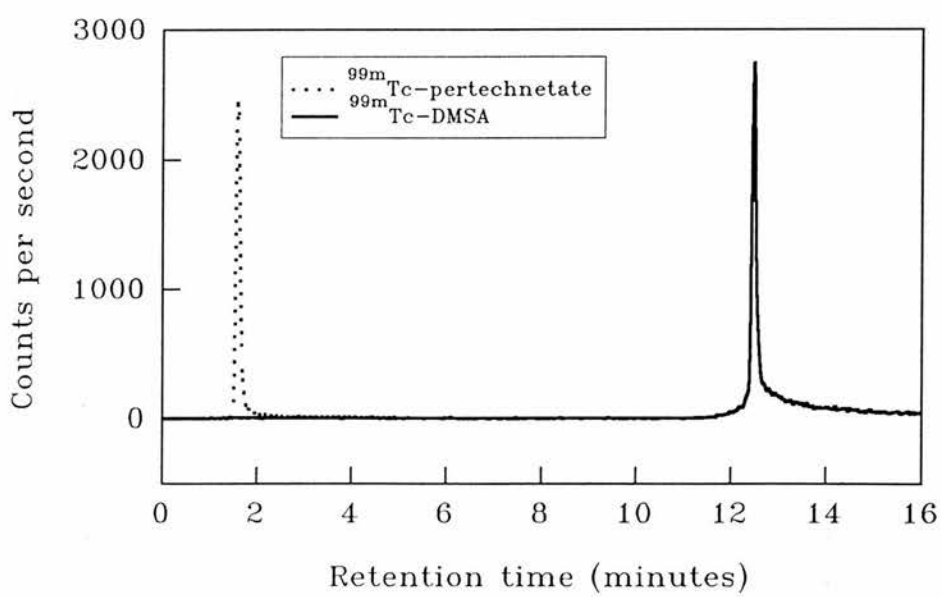

Figure 5.1 *High performance liquid chromatogram of ^{99m}Tc-DMSA on Hypersil-ODS using the conditions of Moretti et al. (1982).*

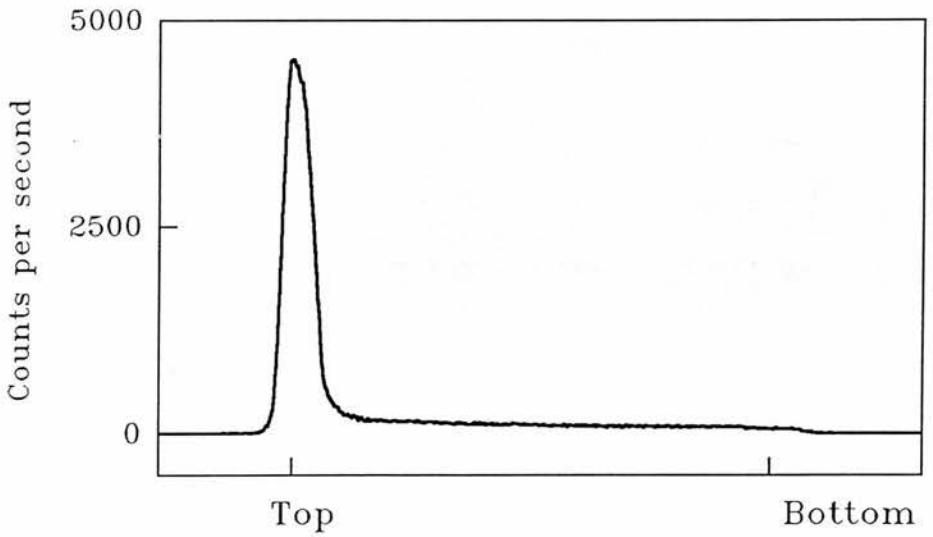

Figure 5.2 *Profile of ^{99m}Tc retained on Hypersil-ODS column.*

Two explanations for the adsorption of ^{99m}Tc at the top of the column can be postulated:

- the ^{99m}Tc-DMSA may contain more than one radiolabelled species, one or more of which are irreversibly bound by Hypersil-ODS under the conditions used in this experiment

- species may have been retained at the top of the column from samples of radiopharmaceuticals injected previously. These species may either retain a proportion of the molecules of ^{99m}Tc-DMSA applied to the column or strip the ^{99m}Tc from a proportion of the ^{99m}Tc-DMSA applied to the column. This possibility could be tested by repeating the experiment with a new Hypersil-ODS column.

To investigate this second possibility, a new Hypersil-ODS column was packed. The first sample to be injected onto this column was ^{99m}Tc-DMSA prepared as above. A chromatogram identical to that shown in Figure 5.1 was obtained. Recovery from the column was 59% which is comparable to that obtained in the earlier experiment. From this result it can be concluded that the adsorption at the top of the column is not caused by

interference from species that have been retained from earlier samples. It is therefore likely that the adsorption can be explained by the presence in ^{99m}Tc-DMSA of more than one species, one or more of which become adsorbed on Hypersil-ODS.

To determine the effect of a lower DMSA/Tc ratio, the experiment was repeated with ^{99m}Tc-DMSA that had been prepared by reconstituting a DMSA kit with Sodium Pertechnetate [^{99m}Tc] Injection that had been obtained from a generator that had not been eluted for 9 days. The eluate therefore contained ^{99}Tc and ^{99m}Tc in a ratio of 98:1. Due to the age of the generator, the radioactive concentration of the eluate was 325 MBq/ml. Reconstituting a DMSA kit with 2 ml of this eluate therefore resulted in ^{99m}Tc-DMSA containing only 650 MBq/2 ml. Nevertheless, this gave a DMSA/Tc ratio of 10^3. When this preparation of ^{99m}Tc-DMSA was analysed as before, a chromatogram identical to that shown in Figure 5.1, i.e. containing a single peak with a retention time of 12.5 minutes, was detected. Recovery from the column was again low at 56%.

Although the poor recoveries from the columns are a major limitation on the credibility of this work, no differences have been detected in ^{99m}Tc-DMSA prepared at DMSA/Tc ratios of 10^4 and 10^3. In routine radiopharmacy practice it is difficult to imagine circumstances that would lead to a ^{99m}Tc generator remaining uneluted for the 9 days that would be required to result in a DMSA/Tc ratio of 10^3.

For many years, several ^{99m}Tc radiopharmaceuticals, including ^{99m}Tc-DMSA, have been prepared in the Radiopharmacy at the Royal Infirmary of Edinburgh by a technique that has become known as "wet labelling" (Millar et al. 1981). In this technique, the radiopharmaceutical kit is reconstituted with Sodium Chloride Injection rather than Sodium Pertechnetate [^{99m}Tc] Injection. For each patient dose required, an aliquot of the reconstituted kit is dispensed into an empty sterile vial. The volume of Sodium Pertechnetate [^{99m}Tc] Injection that contains the appropriate activity of ^{99m}Tc is then dispensed into the vial. The labelling reaction therefore takes place in the patient dose vial rather than in the kit. The term "wet labelling" was used to describe this technique since the ^{99m}Tc is added to a solution rather than a freeze-dried powder. The wet labelling technique offers a number of advantages in routine radiopharmacy practice:

- depending upon the workload in the radiopharmacy and the manner in which ^{99m}Tc radiopharmaceuticals are prepared, it can lead to a controlled, almost production line-like, flow of work

- providing the kit is reconstituted in a standard volume and then a standard volume of the solution is used for the preparation of each patient dose, wet labelling ensures that a standard mass of radiopharmaceutical is contained in each dose. This is good practice, particularly in the preparation of ^{99m}Tc Macroaggregated Albumin Injection when the number of particles that are injected into the patient should be controlled and in situations where measurement of uptake of the radiopharmaceutical is performed e.g. measurement of the renal uptake of ^{99m}Tc-DMSA

- urgently required doses of radiopharmaceuticals can be prepared when requested without the need to use a fresh kit which involves additional expense, or the need to prepare additional doses in anticipation of possible requests which will subject the radiopharmacy staff to unnecessary exposure to radiation

- the principal benefit of this technique is that the ^{99m}Tc for each patient dose is handled only once. When preparing a ^{99m}Tc radiopharmaceutical by reconstituting a kit with the total activity of ^{99m}Tc that is required for all the patient doses requested, the operator is first irradiated by the ^{99m}Tc during kit reconstitution and again while dispensing the individual patient doses from the reconstituted kit. When using the wet labelling technique, only one exposure to the ^{99m}Tc is necessary thereby reducing the radiation dose received by the operator

As this technique is in routine use, I decided to establish the chromatographic behaviour of ^{99m}Tc-DMSA prepared in this way. According to the procedure used routinely, a DMSA kit was reconstituted with 10.5 ml of Sodium Chloride Injection. A 1.0 ml aliquot of the resulting solution was transferred to a sterile nitrogen-filled 10 ml vial (Product Code N46, Amersham). Sodium Pertechnetate [^{99m}Tc] Injection (150 MBq/1.5 ml) was then injected into the vial. As in the first experiment, the previous elution of the generator from which the Sodium Pertechnetate [^{99m}Tc] Injection was obtained had been 24 hours earlier. After standing at room temperature for three hours, the ^{99m}Tc-DMSA was analysed by the HPLC technique described above. As before, a single peak with a retention time of 12.5 minutes and a recovery of 61% was detected. Under these conditions therefore, ^{99m}Tc-DMSA prepared according to Amersham's instructions and the wet labelling procedure are

chromatographically identical. In the Radiopharmacy, ^{99m}Tc-DMSA is prepared each morning by the wet labelling technique and extra doses can be prepared at little additional cost. As I had been unable to show a difference between ^{99m}Tc-DMSA prepared by the two techniques, I decided to use the readily available wet labelled ^{99m}Tc-DMSA in future experiments.

In an attempt to increase the recovery from the column 50% methanol was introduced into the mobile phases used in the previous experiments. As might be anticipated when the organic component of the mobile phase is increased, the retention time of the peak was reduced. A single peak with a retention time of one minute was detected. Recovery from the column was 84%. This was higher than had been observed in the previous experiments but was still too low to be of practical value. Also, a one minute retention time is probably that of an unretained solute which is an unsatisfactory condition for an analytical technique.

Increasing the concentration of the phosphate buffers in the mobile phase from 10 mM to 100 mM had no effect on the chromatogram although the recovery was higher at 72%.

Although the results have not demonstrated that Tc concentration or the use of the wet labelling technique has any effect on radiochemical purity, analysis on Hypersil-ODS has not been found to be an ideal technique due to the significant adsorption of Tc onto the column packing. I therefore decided to investigate other column packing materials that might not exhibit similar adsorptive properties.

5.3 Analysis on Hypercarb

A possible explanation for the adsorption of ^{99m}Tc onto Hypersil-ODS may be the presence of uncapped silanol sites in the stationary phase. At the exceptionally low concentrations of radiolabelled species found in radiopharmaceuticals, typically a few nanograms per ml, the presence of these sites may assume a greater significance than during more common analyses of mixtures when microgram quantities of analytes are present. I therefore decided to investigate Hypercarb as an alternative stationary phase. This HPLC packing material consists of rigid porous graphitised carbon spheres and has not previously been used in the analysis of radiopharmaceuticals.

The potential advantages of Hypercarb are that it a) is not silica-based and is therefore free

from unreacted silanol sites and b) is stable over the complete pH range.

Before attempting to analyse ^{99m}Tc-DMSA on Hypercarb, I decided to investigate the recovery of ^{99m}Tc-pertechnetate since, if this simplest form of Tc is retained, there would be no point in pursuing the use of this column further. A 100 x 5 mm Hypercarb column was therefore equilibrated with a common mobile phase of methanol:water (90:10) at a flow-rate of 1 ml/minute. The equipment described earlier in this chapter was used. A 20 μl sample of Sodium Pertechnetate [^{99m}Tc] Injection (approximately 100 MBq/ml) was injected onto the column and recovery was measured as described in Chapter 3. This procedure was repeated four times. A chromatogram containing a single peak with a retention time of 1.5 minutes was detected. The mean recovery from the column was 101.7 $\pm$ 0.9% (n=5). This result demonstrates that under the conditions used, there is no interaction between Hypercarb and Tc that might interfere with the detection of ^{99m}Tc-pertechnetate impurity during analysis of ^{99m}Tc radiopharmaceuticals. However, this finding cannot be taken as being valid for all mobile phases. It will therefore be necessary to confirm this high recovery with any other mobile phase that shows promise for the analysis of ^{99m}Tc-DMSA.

Using the methanol:water (90:10) mobile phase, ^{99m}Tc-DMSA was injected onto the column. After 20 minutes, no peaks had been recorded and no ^{99m}Tc was detected in the eluate. When the column was removed from the instrument and scanned, all the ^{99m}Tc was detected at the top.

Various mobile phases were then investigated in an effort to resolve the problem of 100% retention on the column. Experiments were performed at least one day apart to allow sufficient time for any ^{99m}Tc retained on the column from the previous experiment to decay to an insignificant activity. This ensured that recovery measurements would not be affected by the elution of any ^{99m}Tc adsorbed during the previous experiment.

- The effect of increasing the organic component of the mobile phase was investigated by using acetonitrile:dioxan (90:10). These mobile phase components were chosen since they have been found useful when used with Hypercarb in other situations (Shandon product literature)

- The effect of a mobile phase of higher pH was investigated by using methanol:100 mM ammonium carbonate (90:10). The pH of 100 mM

ammonium carbonate is 7.9.

- Methanol:100 mM acetic acid was tried to determine the effect of reducing pH. Amersham International quote a pH range of 2.3 - 3.3 for ^{99m}Tc-DMSA. This mobile phase therefore had the attraction of being in the pH range of the radiopharmaceutical as 100 mM acetic acid has a pH of 2.7. The prospect of analysing the radiopharmaceutical using a mobile phase of similar pH is attractive since it could be envisaged that alteration of pH during analysis could bring about an alteration in the composition of the radiopharmaceutical especially when it is known that pH plays an important part in the labelling reaction

- The effect of a low pH and a higher proportion of organic component was investigated using methanol:100 mM acetic acid:dioxan (72:8:20)

With each of these mobile phases, no peaks were detected and no ^{99m}Tc was recovered from the column. It therefore appears that Hypercarb has an exceptionally high affinity for the radiolabelled species that are present in ^{99m}Tc-DMSA. In view of this complete adsorption onto Hypercarb regardless of the mobile phase used, I decided not to pursue this stationary phase further.

5.4 Analysis on PLRP-S

In the course of an incomplete and unpublished investigation into the analysis of ^{99m}Tc-DMSA by HPLC, Theobald (personal communication) has reported the detection of interesting multi-peak chromatograms when using PRP-1 (Hamilton) and PLRP-S (Polymer Labs) columns. These columns are similar in that they are both packed with a reversed-phase macroporous copolymer of styrene and divinylbenzene. In my quest for an HPLC method for anaylsing ^{99m}Tc-DMSA, I therefore decided to investigate the use of the PLRP-S column.

As in my previous experiments, the first task was to carry out a preliminary assessment of the suitability of the PLRP-S column by measuring the recovery of ^{99m}Tc-pertechnetate. As before, this was carried out using a mobile phase of methanol:water (90:10). A single peak was detected at a retention time of 7.5 minutes and with a satisfactory recovery of

101.0 ± 2.3% (n=5).

The mobile phase used by Theobald consisted of a citrate/phosphate/borate/HCl buffer containing 1% methanol with most of the interesting results being obtained at pH2-3. Hydrochloric acid is not recommended for use in HPLC mobile phases as it attacks the stainless steel components of the instrument. I therefore considered the use of this mobile phase inadvisable. As a more acceptable alternative I decided to achieve a low pH through the use of an acetate buffer. The column was therefore equilibrated at 1 ml/minute with a mobile phase of 100 mM acetate buffer pH3:acetonitrile (90:10). When ^{99m}Tc-DMSA was injected onto the column, no peaks were detected and no ^{99m}Tc was detected in the eluate. When the column was removed from the instrument and scanned, all the ^{99m}Tc was detected in the guard column and at the top of the analytical column. The profile of ^{99m}Tc in the column is shown in Figure 5.3.

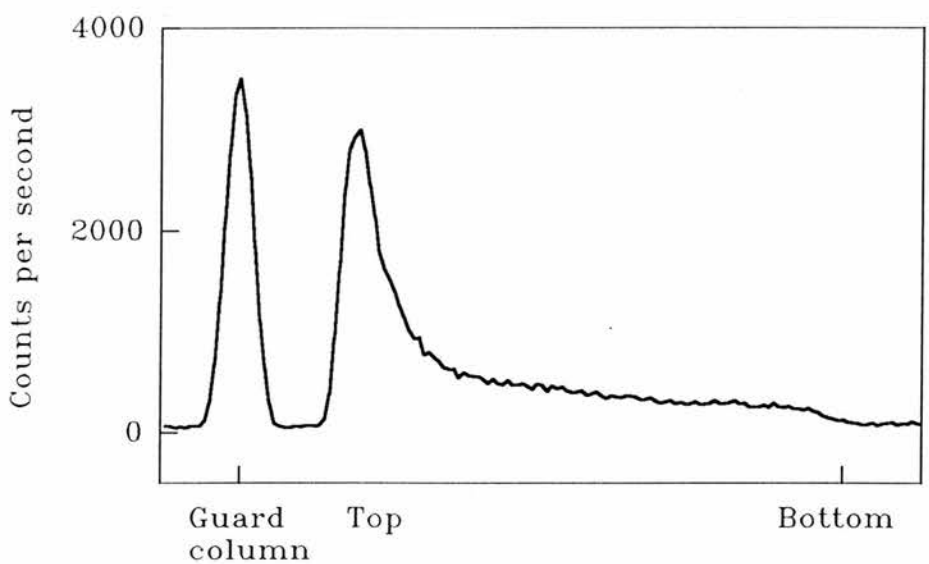

Figure 5.3 *Profile of ^{99m}Tc retained on PLRP-S column eluted with 100 mM acetate buffer pH3: acetonitrile (90:10).*

As an alternative means of achieving a low pH, I decided to investigate the use of citric acid which was one of the acids present in Theobald's mobile phase. Using a mobile phase of 100 mM citric acid (pH2.2):acetonitrile (90:10) a chromatogram containing three peaks

was detected. The peaks had retention times of 4.9, 6.5 and 16.1 minutes and the chromatogram is shown in Figure 5.4. Recovery was only 58% but this was the first occasion on which I had detected a multi-peak chromatogram with ^{99m}Tc-DMSA. The column was removed from the instrument and scanned as before. A profile of activity identical to that shown in Figure 5.3 was recorded showing that the ^{99m}Tc retained in the column was in the guard column and at the top of the analytical column. Various alterations to the mobile phase such as increasing the proportion of acetonitrile and raising the pH of the citric acid were introduced in attempts to improve recovery but none were successful. Recovery was variable and never found to be greater than 67%.

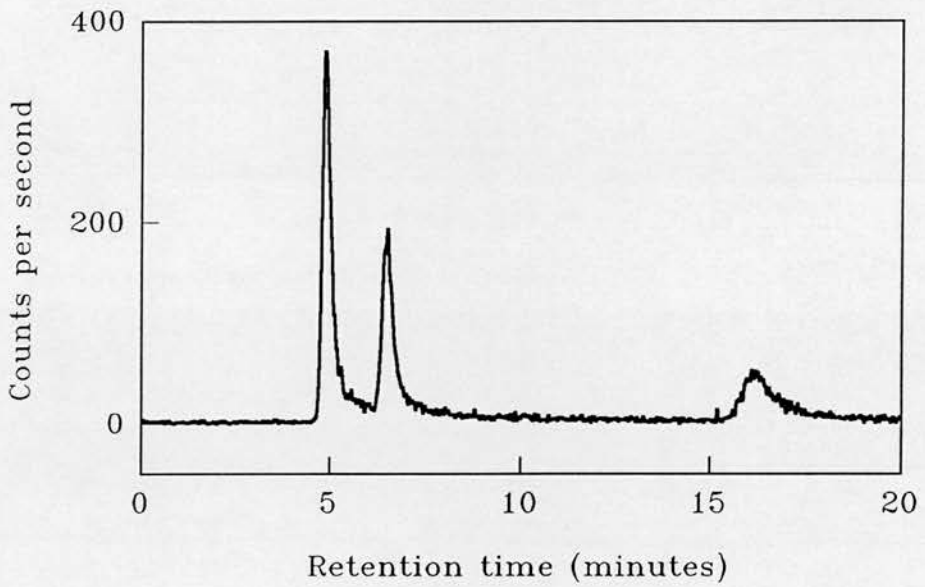

Figure 5.4 *High performance liquid chromatogram of ^{99m}Tc-DMSA on PLRP-S eluted with a mobile phase of 100 mM citric acid: acetonitrile (90:10).*

I therefore decided to investigate the effect of a mobile phase with an alkaline pH. This might be expected to bring all species off the column with the retention time of an unretained solute since, at high pH, the carboxylic acid groups on DMSA would be completely ionised. There would however still be the possibility of interaction between the mercapto groups and the column packing. A mobile phase of acetonitrile:100 mM ammonia (70:30) was used and samples were injected onto the column at 5, 30, 60 and 90 minutes after preparation of the ^{99m}Tc-DMSA. Each chromatogram contained two peaks

with retention times of 88 and 158 seconds (see Figure 5.5) and recoveries of 107%, 114%, 111% and 115% respectively. Although the chromatograms were not of practical value since they suggested the presence of only two species whereas the previous work had revealed at least three, this was the first occasion on which recovery had not been significantly less than 100%. However, recovery of consistently greater than 100% was an unexpected phenomenon that required further investigation.

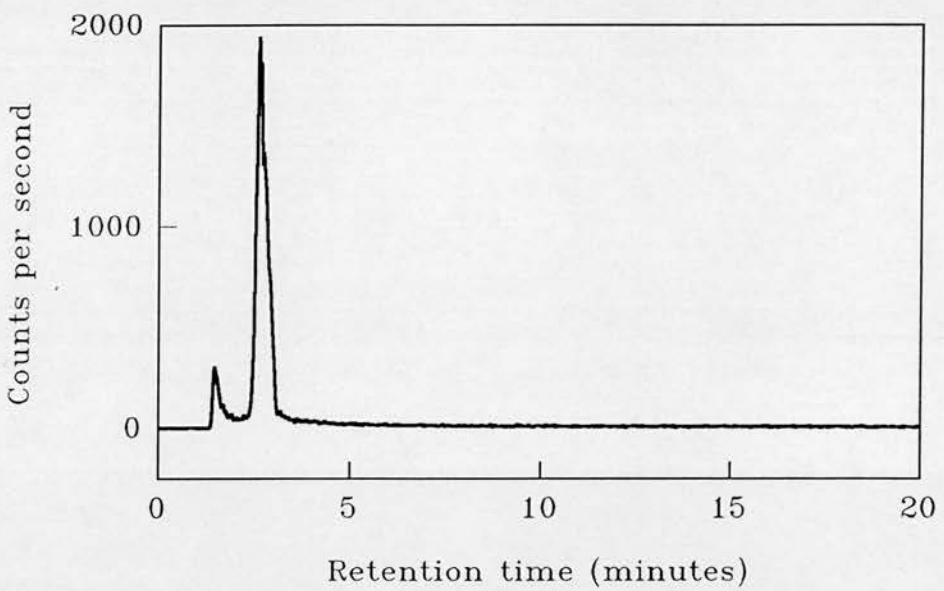

Figure 5.5 *High performance liquid chromatogram of ^{99m}Tc-DMSA on PLRP-S eluted with a mobile phase of acetonitrile: 100 mM ammonia (70:30).*

5.5 Investigation of recoveries of greater then 100%

Since it is impossible for more ^{99m}Tc to be eluted from the column than was applied, a recovery measurement of greater than 100% must be the result of a technical artifact. Several possibilities that could account for the phenomenon were examined.

The development of a fault in the 20 μl pipette used in the recovery measurement could have led to less than 20 μl of sample being present in the standards against which the column eluate was counted. This possibility was investigated by recalibration of the 20 μl pipette against the Rheodyne 20 μl sample loop as described in Chapter 3.4. The

calibration was not found to have changed. This did not therefore explain the measurement of recoveries greater than 100%.

The counting standards used in the recovery measurement technique were made up in water whereas the column eluates were 70% acetonitrile. It is possible that the gamma-rays from ^{99m}Tc are attenuated less by acetonitrile than by water. For the same activity, the count-rate measured from an eluate would therefore be higher than that from a standard. This would lead to the calculation of a recovery of greater than 100%. This possibility was investigated by pipetting 20 μl of sodium pertechnetate [^{99m}Tc] solution into each of six vials. The contents of three vials were made up to 30 ml with water and mixed thoroughly. The contents of the other three vials were made up to 30 ml with acetonitrile:water (70:30) and mixed thoroughly. The count-rate from each vial was measured with the equipment used in the recovery measurements. The mean count-rate from the vials containing acetonitrile:water was 1.4% higher than that from the vials containing water. Although this small difference would lead to measurement of a recovery of greater than 100%, it is not great enough to explain the recoveries of approximately 110% that were observed. Also, the error introduced by attenuation differences could be avoided by preparing standards in mobile phase.

^{99m}Tc-DMSA has been shown to adsorb onto the inside surface of glass vials into which it has been dispensed (Millar 1984). If a similar effect were occurring on the glass capillary tube of the 20 μl pipette that was used to prepare the standards used in the recovery measurement technique, then the amount of ^{99m}Tc transferred to the standard vials would be less than assumed. The low count-rates detected from the standards would therefore result in the calculation of a recovery of greater than 100%. To determine if this provides an explanation for the high recoveries, ^{99m}Tc-DMSA was drawn into the 20 μl pipette which was then placed on the platform of the detector used in the recovery measurement technique. The count-rate from the ^{99m}Tc in the pipette was recorded. The contents were then expelled and the count-rate from the empty pipette was recorded. Approximately 1% of the original activity was found to be retained in the emptied pipette. Adsorption in the pipette was not therefore responsible for the recovery measurements of greater than 100%.

With similar reasoning in mind, I considered that the ^{99m}Tc-DMSA eluted from the column might become adsorbed on the inside surfaces of the glass collection vial, perhaps as a consequence of the high pH of the mobile phase. Assuming that a similar effect did not occur with the standards which were made up in water, attenuation of the gamma-rays

emitted from the vial of eluate would be lower than from the standard vials. This effect would be particularly significant if adsorption was predominantly on the base of the vial since this is the part of the vial nearest to the detector. This situation can be envisaged since the ^{99m}Tc was eluted from the column in the first few millilitres of mobile phase that were collected and would therefore have been in intimate contact with the base of the vial. To explore this possibility, ^{99m}Tc-DMSA was injected onto the column and the eluate was collected in the standard manner. After collection of approximately 10 ml, the vial was removed from the instrument and the count-rate from it was measured. The solution was then poured from the vial and the count-rate from the emptied vial was recorded and found to be approximately 1% of that from the full vial. Residual liquid in the vial was probably responsible for this activity but I decided not to dry the vial in case adsorbed ^{99m}Tc was wiped from the glass. However, the low residual activity was not sufficient to explain the recoveries of greater than 100% that were measured.

Adsorption of ^{99m}Tc-DMSA onto the sample loop during the loading of the valve would be a possible explanation for recoveries of greater then 100% if the adsorbed ^{99m}Tc was subsequently removed by the mobile phase when the valve was switched to the "Inject" position. To investigate this possibility, the radioactive concentration of a ^{99m}Tc-DMSA solution before and after it had been passed through the sample loop was measured in the following experiment. The sample loop of the Rheodyne valve was flushed with water. A 1 ml syringe was filled with a ^{99m}Tc-DMSA solution and the needle supplied for loading the valve was fitted to the syringe. Approximately 0.5 ml of the ^{99m}Tc-DMSA solution was transferred to a weighed sample tube. With the valve in the "Load" position, the remaining 0.5 ml was passed through the sample loop and collected in a second weighed sample tube. Both tubes were reweighed and the weight of solution in each was calculated. The count-rate from each tube was measured by the technique used for recovery measurements. The count-rate per gram of liquid in each vial was then calculated. This experiment was carried out three times. On each occasion, the liquid that had not been passed through the loop had a higher radioactive concentration than the liquid that had. In the three experiments, the mean count-rates per gram of the samples that had not been passed through the valve were 13% (range 10% to 15%) higher than those of the samples that had not been through the valve. These findings provide an explanation for the measurement of recoveries greater than 100%. As these excessive recoveries were only seen when a mobile phase of high pH was used, a possible explanation for the effect is that at the low pH of ^{99m}Tc-DMSA adsorption onto the sample loop occurs during loading of the valve. When the loop is switched into the flow of a mobile phase with a high pH, the adsorbed ^{99m}Tc-DMSA is

washed off the walls of the loop and into the column along with the ^{99m}Tc-DMSA that is in solution in the loop. More ^{99m}Tc than would be present in 20 μl of sample would therefore be applied to the column.

This phenomenon raises the question of the validity of all the recovery measurements made so far in this work. The interesting chromatograms have all been obtained using acidic mobile phases. It could therefore be the case that adsorption in the stainless-steel loop has occurred but with the mobile phase having a pH similar to that of the ^{99m}Tc-DMSA, the adsorbed ^{99m}Tc was not washed onto the column resulting in the measurement of low recovery. While this effect has undoubtedly contributed to the low recoveries that have been detected, column scanning has revealed the presence of ^{99m}Tc in the guard column and at the top of the analytical column. Adsorption in the sample loop cannot therefore provide a complete explanation for low recoveries.

As a possible means of overcoming the phenomenon of adsorption in the valve, I decided to try replacing the stainless-steel loop with one made from PEEK tubing. The rationale for this approach was that PEEK is reported to be a particularly inert material and should therefore exhibit low adsorption characteristics. A 40 cm length of PEEK tubing (internal diameter 0.25 mm) was cut and fitted to the valve with PEEK fittings (Anachem) in place of the stainless steel loop. This provided a PEEK loop with a nominal volume of 20 μl. The experiment used to detect adsorption in the stainless-steel loop was repeated. The mean count-rate per gram (n = 5) from the samples of ^{99m}Tc-DMSA that were not passed through the loop were only 2% (range 0 to 5%) higher than the mean count-rate per gram from the samples that were passed through the loop. This much reduced adsorption in comparison to the stainless-steel loop demonstrates that PEEK tubing is the more suitable material for use with ^{99m}Tc-DMSA. In view of these findings, I also replaced the length of tubing between the valve and the column and the column outlet tubing which constituted the detector cell with PEEK tubing to minimize adsorption throughout the system.

5.6 Effect of PEEK tubing on recovery

With the sample loop, valve-to-column connection and column outlet/detector cell all constructed from PEEK tubing, the column was equilibrated with a mobile phase of 100 mM citric acid/acetonitrile (85:15) as before and ^{99m}Tc-DMSA was injected. The chromatogram was identical to that obtained previously and shown in Figure 5.4.

Recovery was only 53% which was comparable to the recoveries obtained previously. These results confirmed that although the adsorption in the sample loop is an interesting observation, it does not explain the low recoveries that have been seen throughout this work. Although adsorption in the loop did not provide a solution to the problem of low recoveries, I decided to retain the PEEK components for the remaining work on ^{99m}Tc-DMSA since they eliminated a possible source of error.

5.7 Identification of the site of adsorption

Figure 5.3 demonstrates that ^{99m}Tc becomes adsorbed in the guard column and at the top of the column but does not indicate the exact site of the adsorption. Having identified a stainless steel component as a site of adsorption in the injection valve, I considered it possible that the adsorption in the column was on the stainless-steel inlet frits. This would explain the localisation of ^{99m}Tc in the guard column and at the top of the analytical column. If this were the case, it might be possible to replace the stainless-steel inlet frit with one made from a more inert material.

The following experiment was therefore carried out to identify the site of adsorption. The guard column was removed and the analytical column was connected directly to the Rheodyne valve. Using the citric acid/acetonitrile mobile phase as before, a sample of ^{99m}Tc-DMSA was injected onto the column. A chromatogram identical to those obtained previously was recorded. Once the third peak of the chromatogram had been detected, the pump was switched off and the column was removed from the instrument and scanned as described in chapter 3.5. A single peak of activity was detected at the top of the column as before. The column top-fitting, which incorporates the stainless-steel inlet frit, was then carefully removed from the column and replaced temporarily by a top-fitting from an identical column. The column was then re-scanned. The profiles of activity along the column before and after replacement of the top-fitting are shown in Figure 5.6. In both cases, a single peak of activity was detected at the top of the column. On each profile, a region of interest was set around the peak of activity and the total counts in the peak were recorded. After correction for background and the radioactive decay that occurred during the time that elapsed between the two scans being performed, the counts from the column after removal of the top-fitting were 86% of the counts that were detected with the original top-fitting in place. This suggests that some adsorption might have been on the top-fitting which includes the inlet frit. However, it is possible that column packing material onto

which some of the ^{99m}Tc had adsorbed was in the top-fitting, perhaps pressed into the underside of the frit. Despite this small anomaly, this experiment demonstrates that adsorption is predominantly on the column packing material, not the stainless steel inlet frit.

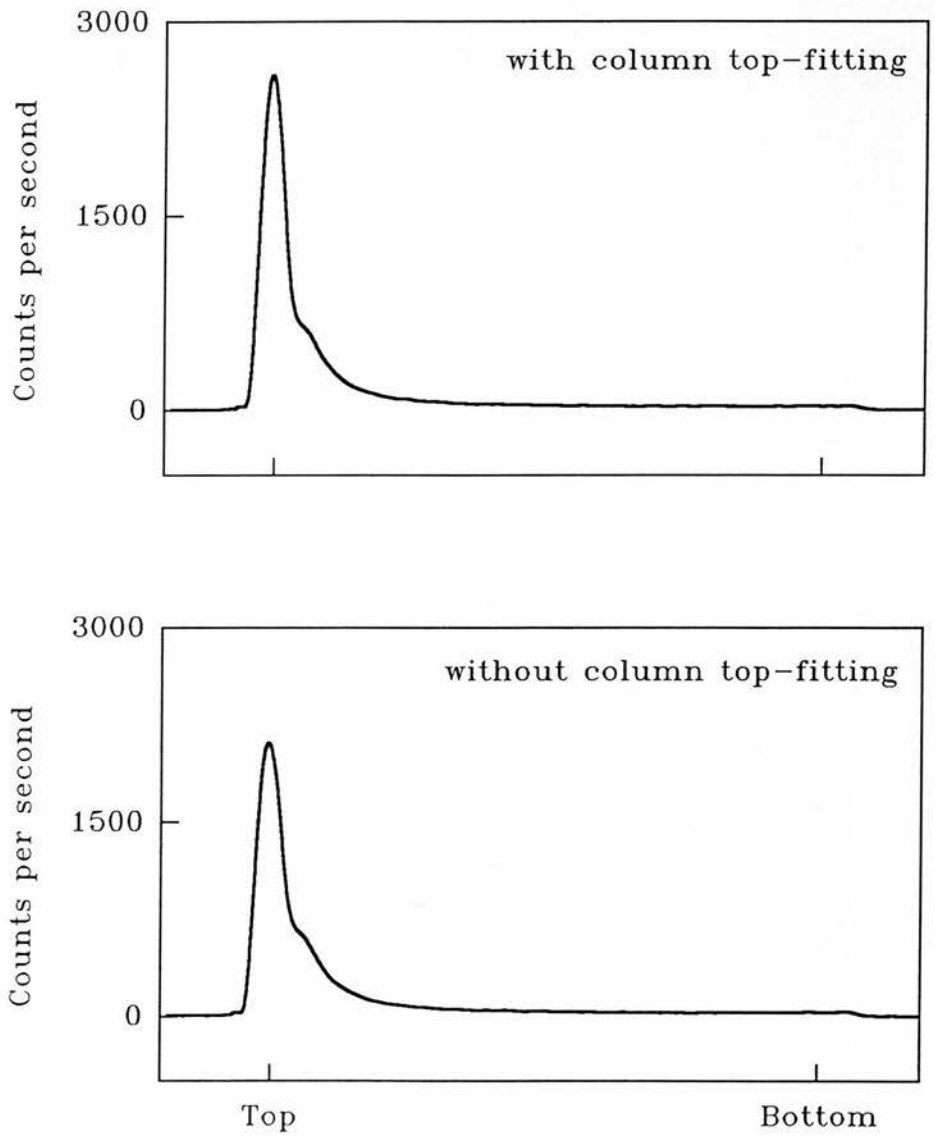

Figure 5.6 *Profile of ^{99m}Tc along a PLRP-S column with and without the column top-fitting.*

5.8 Identification of the presence of ^{99m}Tc(V)-DMSA

At this stage of my investigations, Blower et al. (1991) published work on the chemical identity of pentavalent ^{99m}Tc-DMSA (^{99m}Tc(V)-DMSA). This radiopharmaceutical has been shown to be suitable for imaging of medullary carcinoma of the thyroid (Ohta et al. 1984). It differs from the kidney-imaging ^{99m}Tc-DMSA in that the ^{99m}Tc is in the pentavalent state. There is some doubt as to the valency of the ^{99m}Tc in the kidney imaging radiopharmaceutical; Ikeda et al. (1976) attributed a valency state of 3 while Westera et al. (1985) have attributed a valency state of 4. Most reports in the literature assume the tri-valent state for ^{99m}Tc-DMSA, therefore from now on I will refer to the kidney imaging agent as ^{99m}Tc(III)-DMSA to distinguish it from the pentavalent species. However, when referring to ^{99m}Tc-DMSA of indeterminate composition, I will continue to use the description ^{99m}Tc-DMSA.

In the course of their work, Blower et al. analysed ^{99m}Tc(V)-DMSA by HPLC using gradient elution of a PRP-1 column (Hamilton) with a 0.1% trifluoroacetic acid in water/0.1% trifuoroacetic acid in acetonitrile mobile phase. This technique resulted in a chromatogram containing three peaks which were attributed to the *syn-endo, syn-exo* and *anti* isomers of the ^{99m}Tc(V)-DMSA complex. Could the three peaks detected in my PLRP-S/100 mM citric acid:acetonitrile (85:15) system be due to the presence of ^{99m}Tc(V)-DMSA in what I have up to now though of as ^{99m}Tc(III)-DMSA?

To answer this question, the PLRP-S column was equilibrated with the 100 mM citric acid:acetonitrile (85:15) mobile phase as before. ^{99m}Tc(V)-DMSA was prepared according to the following method of Westera et al. (1985). A DMSA kit (Amersham) was reconstituted with 0.4 ml of Sodium Bicarbonate 8.4% Injection. After dissolution of the freeze-dried powder, Sodium Pertechnetate [^{99m}Tc] Injection (400 MBq/2.1 ml) was injected into the kit. The solution was incubated at room temperature for 30 minutes. A 20 μl sample of the ^{99m}Tc(V)-DMSA was injected onto the column and a chromatogram identical to that obtained with ^{99m}Tc-DMSA was detected. The chromatogram is shown in Figure 5.7. However, whereas recovery with ^{99m}Tc-DMSA was typically 50%, recovery from ^{99m}Tc(V)-DMSA was 92%. This result suggests strongly that the chromatogram I have been detecting was due to the presence of ^{99m}Tc(V)-DMSA in what I had considered to be ^{99m}Tc(III)-DMSA.

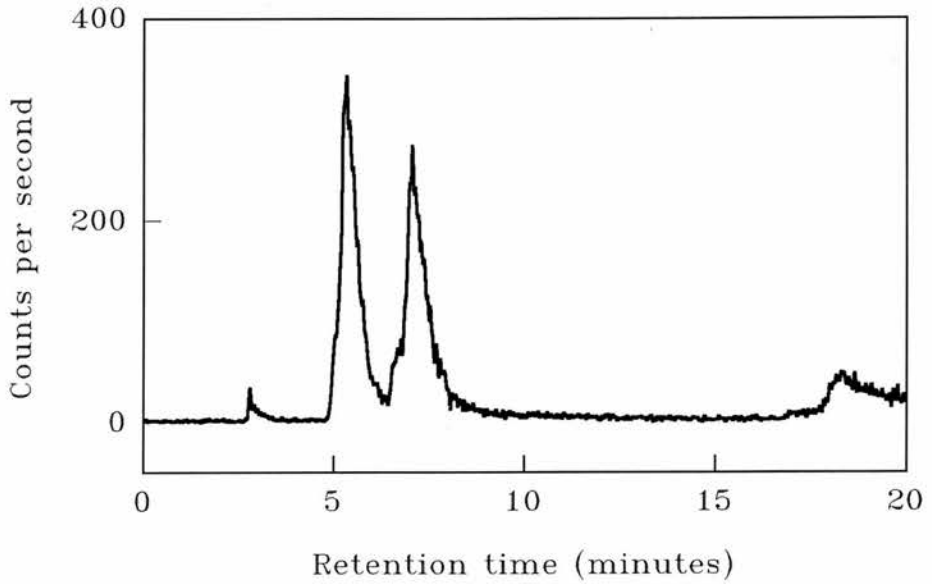

Figure 5.7 *High performance liquid chromatogram of ^{99m}Tc(V)-DMSA on a PLRP-S column eluted with 100 mM citric acid: acetonitrile (90:10).*

To investigate this possibility further, I decided to analyse ^{99m}Tc-DMSA and ^{99m}Tc(V)-DMSA using the thin-layer chromatographic technique described by Ohta et al. (1984) for the analysis of ^{99m}Tc(V)-DMSA. This analysis is carried out using silica gel 60 plates (Merck) and a mobile phase of butan-1-ol/acetic acid/water (3:2:3). The plates were spotted 30 minutes after preparation of the radiopharmaceuticals. The mobile phases were allowed to travel 7 cm then the plates were dried and scanned as described in Chapter 3.7. The profile of activity along each plate is shown in Figure 5.8. The chromatogram of ^{99m}Tc(V)-DMSA shows a single peak of activity at Rf 0.6 while the chromatogram of the ^{99m}Tc(III)-DMSA shows a wide peak around the origin and a second well-defined peak at Rf 0.6. This experiment provides further evidence of the presence of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA that has been prepared by the wet labelling technique.

The B.P. (1993) method for measuring the radiochemical purity of ^{99m}Tc-DMSA, or ^{99m}Tc-succimer as it is now called in the B.P., specifies the use of thin-layer chromatography with ITLC/SG (Gelman) as the stationary phase and butan-2-one as the solvent. In this chromatographic system, the B.P. states that the ^{99m}Tc-DMSA complex remains on the line of application and pertechnetate ion migrates near to the solvent front. ^{99m}Tc-pertechnetate

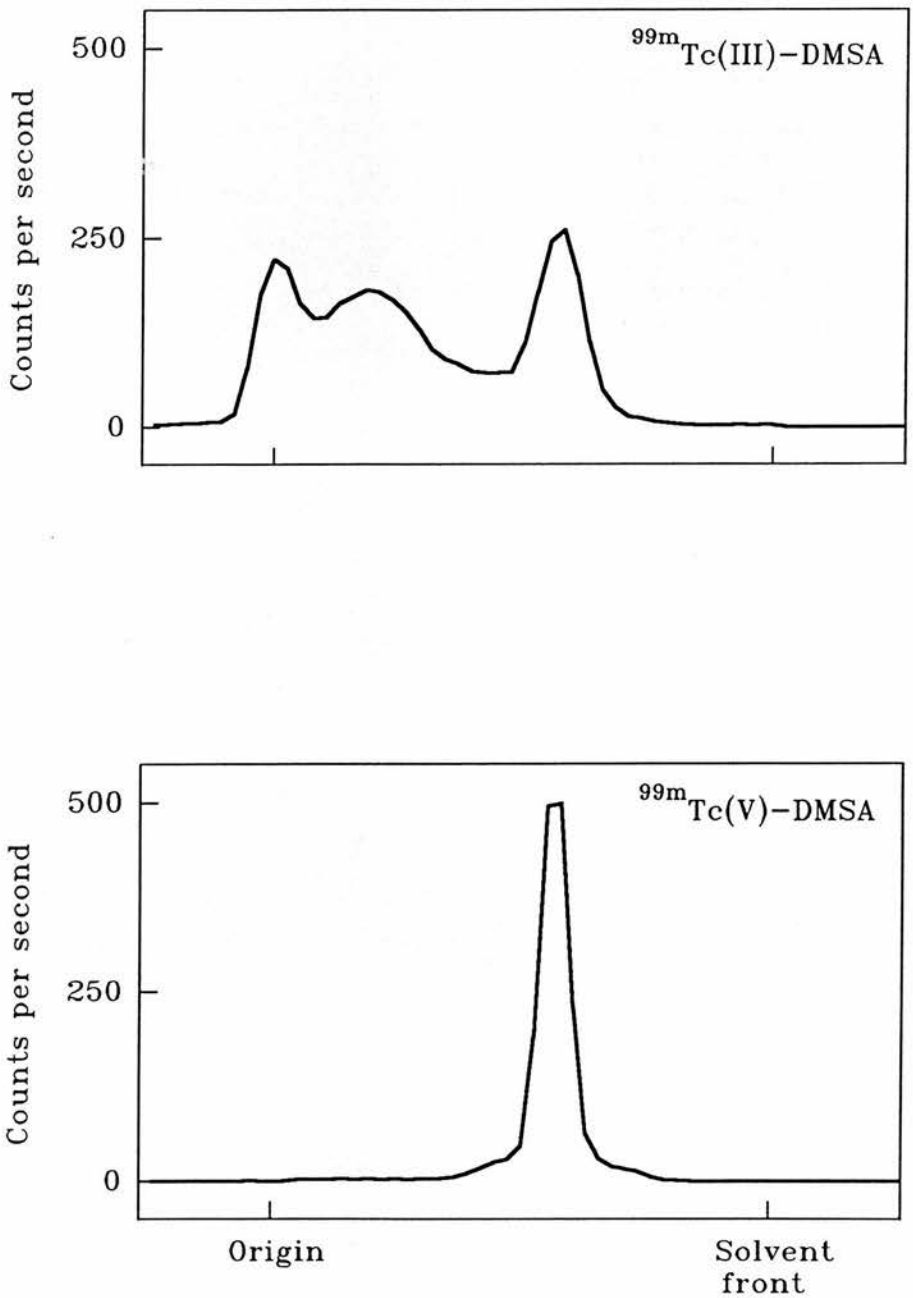

Figure 5.8 *Thin-layer chromatograms of ^{99m}Tc(III)-DMSA and ^{99m}Tc(V)-DMSA*

is therefore considered to be the only potential impurity. However, I have demonstrated that ^{99m}Tc(V)-DMSA can also be present as an impurity in ^{99m}Tc-DMSA. I therefore tested the behaviour of ^{99m}Tc(V)-DMSA under these chromatographic conditions and found that all the ^{99m}Tc remains at the origin. The conclusion from these findings is therefore that the B.P. technique for determining the radiochemical purity of ^{99m}Tc-DMSA is inadequate as it is incapable of detecting ^{99m}Tc(V)-DMSA when it is present as an impurity.

5.9 Effect of alternative acidic components in the mobile phase

The three peak chromatogram that is attributable to ^{99m}Tc(V)-DMSA has been obtained using a mobile phase of containing citric acid and acetonitrile. Blower et al.(1991) achieved their three peak chromatogram with a mobile phase containing trifluoroacetic acid and acetonitrile. It is possible, therefore, that a low pH is the important factor and the means of obtaining this low pH is less important. This was tested by preparing ^{99m}Tc-DMSA by wet labelling and, 10 minutes after preparation, analysing the solution using the following mobile phases: 100 mM citric acid:acetonitrile (85:15), 100 mM acetic acid:acetonitrile (85:15) and 0.1% trifluoroacetic acid:acetonitrile (85:15). The pH of the acidic components of these mobile phases were 2.0, 2.6, and 1.9 respectively. All three mobile phases gave a three peak chromatogram. These are shown in Figure 5.9. This experiment demonstrates that citric acid has no particular properties that lend it to the analysis of ^{99m}Tc-DMSA. The low pH that is required can be achieved through the use of a number of acids.

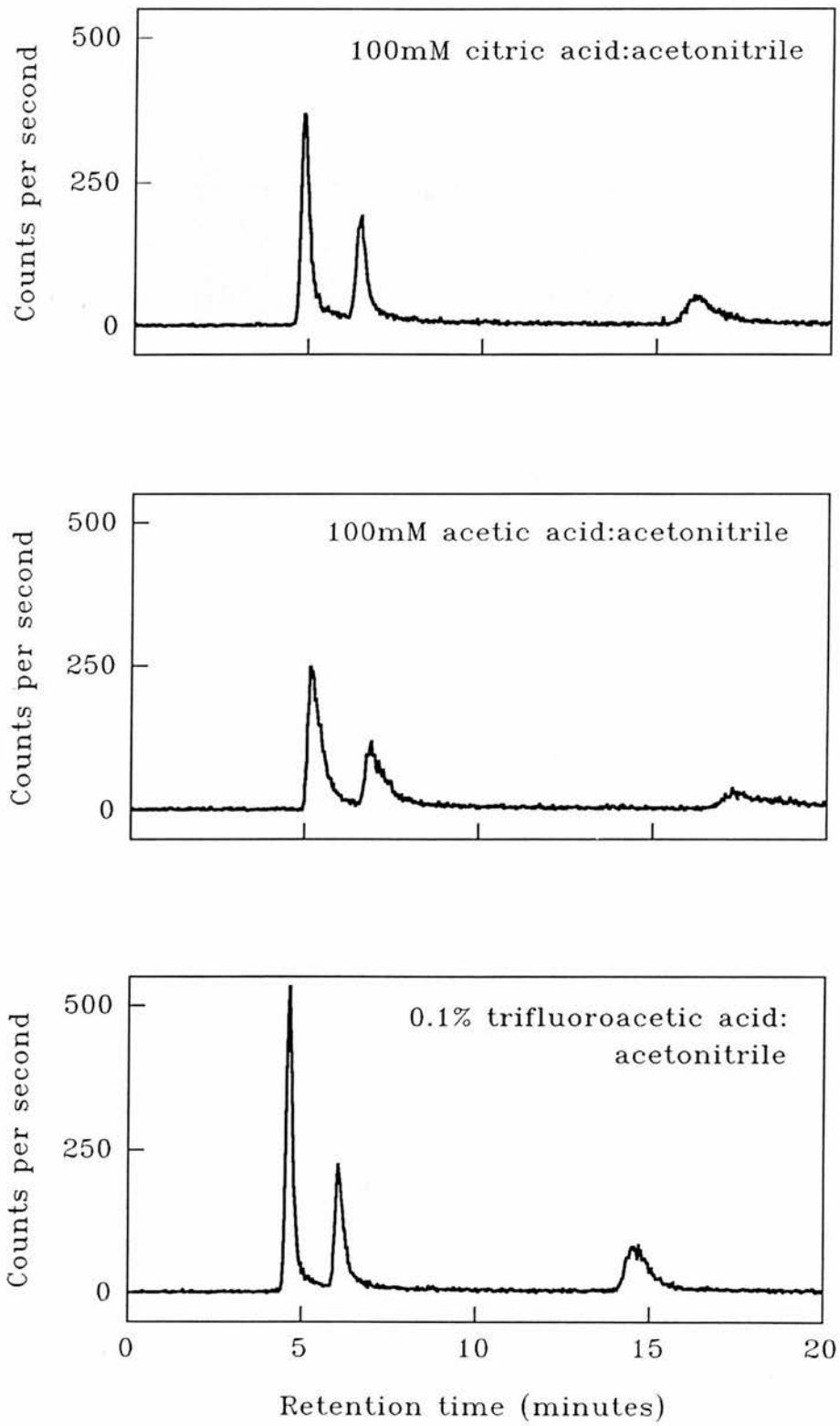

Figure 5.9. *High performance liquid chromatograms of ^{99m}Tc-DMSA using different mobile phases.*

5.10 Effect of the wet labelling technique on the formation of ^{99m}Tc(V)-DMSA

The presence of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA has not been reported in the literature. I therefore decided to investigate if this phenomenon is a consequence of the wet labelling technique that I have used for preparation of the radiopharmaceutical. ^{99m}Tc-DMSA was therefore prepared by both the Amersham recommended technique, i.e. by injecting Sodium Pertechnetate [^{99m}Tc] Injection directly into a DMSA kit, and the wet labelling technique. Thirty minutes after preparation, the two preparations were analysed by both the HPLC and TLC techniques described previously. The chromatograms from the HPLC are shown in Figure 5.10 and those from the TLC are shown in Figure 5.11. Both chromatographic techniques demonstrate the presence of ^{99m}Tc(V)-DMSA in the radiopharmaceutical prepared by the wet labelling technique but the absence of ^{99m}Tc(V)-DMSA in the one prepared by the manufacturer's technique. It can therefore be concluded that wet labelling is responsible for the presence of the ^{99m}Tc(V)-DMSA complex in ^{99m}Tc-DMSA.

To prepare ^{99m}Tc(V)-DMSA as a radiopharmaceutical in its own right, a DMSA kit for the preparation of ^{99m}Tc(III)-DMSA is used but the pH of the reaction mixture is altered. Whereas the ^{99m}Tc(III)-DMSA complex is formed at a pH of 2-2.5, the ^{99m}Tc(V)-DMSA complex is formed if the pH of the reaction mixture is raised to 10. In routine practice, this higher pH is achieved by dissolving the contents of a DMSA kit in Sodium Bicarbonate 8.4% Injection before addition of the Sodium Pertechnetate [^{99m}Tc] Injection. I therefore measured the pH of ^{99m}Tc-DMSA that had been prepared by wet labelling to establish if this technique results in a solution of higher than normal pH which might explain the presence of ^{99m}Tc(V)-DMSA in the radiopharmaceutical. However, the pH was found to be 2.5. Modification of the reaction mixture pH is not therefore the explanation for the formation of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA when preparation is carried out by the wet labelling technique.

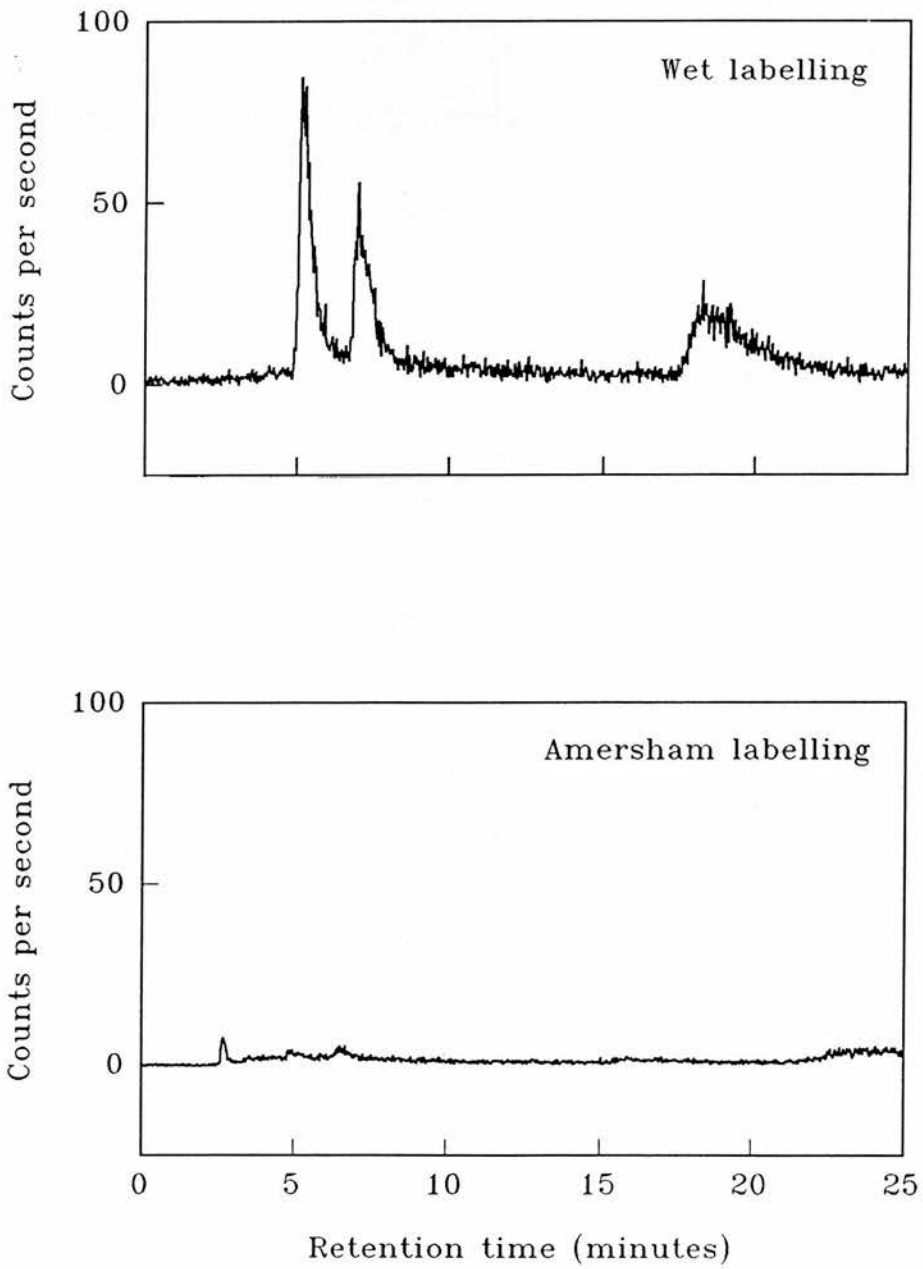

Figure 5.10 *High performance liquid chromatograms of ^{99m}Tc-DMSA 30 minutes after preparation by the wet labelling and Amersham techniques.*

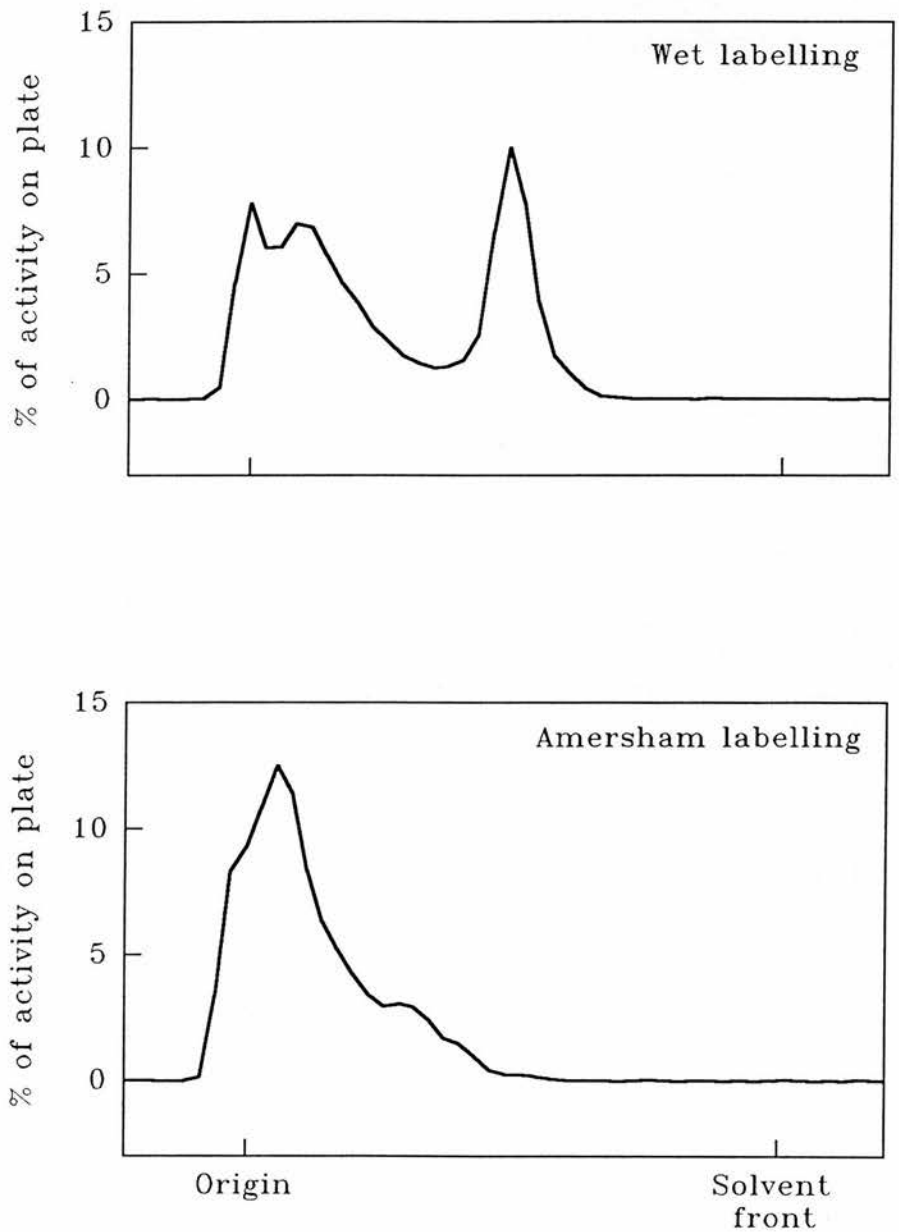

Figure 5.11 *Thin-layer chromatograms of ^{99m}Tc-DMSA 30 minutes after preparation by the wet labelling and Amersham techniques.*

5. Radiochemical Purity of ^{99m}Tc-DMSA Injection

The principal differences between the wet and manufacturer's labelling techniques are that:

- in the manufacturer's technique, the Tc is present as the freeze-dried components in the kit are dissolving

- when the manufacturer's technique is used, the concentration of DMSA in the reaction mixture is between 0.125 and 1 mg/ml depending on the volume of Sodium Pertechnetate [^{99m}Tc] Injection with which the kit is reconstituted. In contrast, wet labelling results in a lower DMSA concentration of 0.04 mg/ml. This difference in concentration also applies to the other constituents of the DMSA kit i.e. the stannous chloride, ascorbic acid and inositol

To test the significance of the first of these differences, ^{99m}Tc-DMSA was prepared in two ways. In the first, which is strictly according to the instructions published by Amersham, Sodium Pertechnetate [^{99m}Tc] Injection (1.5 GBq/6 ml) was injected into a DMSA kit. This represents a situation in which the solution of ^{99m}Tc is added directly to the freeze-dried powder in the kit. In the second, a DMSA kit was reconstituted with 5 ml of Sodium Chloride Injection. Sodium Pertechnetate [^{99m}Tc] Injection (1.5 GBq/1 ml) was then injected into the kit. This represents a modification of the wet labelling technique in which the solution of ^{99m}Tc is added to a solution of the kit contents. The 1.5 GBq of ^{99m}Tc and 6 ml final volume were used since they are the maximum activity and volume recommended by Amersham for the reconstitution of the kit. The solutions were incubated at room temperature for 10 minutes after addition of the ^{99m}Tc. This is the incubation time recommended by Amersham. Analysis of the solutions was then carried out by HPLC using the standard PLRP-S/100 mM citric acid:acetonitrile system. The experiments were performed on separate days to allow time for decay of ^{99m}Tc retained on the column from the first. Both solutions produced the 3 peak chromatogram that has been described previously. Recoveries from the column were comparable at 17.7% and 17.1% respectively. It can be concluded from this experiment that the way in which the ^{99m}Tc and kit contents are mixed does not influence the formation of ^{99m}Tc(V)-DMSA in the radiopharmaceutical. When the DMSA kit is reconstituted at the maximum activity and volume specified by Amersham, 17% of the ^{99m}Tc is present in the form of an impurity after the recommended 10 minute incubation time. This could not be considered a satisfactory product, although in practice, the administration of ^{99m}Tc-DMSA to a patient within 10 minutes of its preparation is extremely unlikely. It would therefore appear that

the factor which influences the presence of ^{99m}Tc(V)-DMSA is the concentration of the reaction mixture. The wet labelling technique involves reconstituting the DMSA kit to a volume of 10.5 ml and then diluting 1.0 ml of this solution to 2.5 ml. This is comparable to diluting the DMSA kit to a volume of 26.3 ml. To demonstrate conclusively that concentration is the factor that influences the level of ^{99m}Tc(V)-DMSA, it would be necessary to compare ^{99m}Tc-DMSA prepared by the standard wet labelling technique with ^{99m}Tc-DMSA prepared by reconstituting a DMSA kit with 26.3 ml of Sodium Pertechnetate [^{99m}Tc] Injection. Unfortunately this experiment is impossible since the DMSA kit is supplied in a 10 ml vial.

5.11 Effect of incubation time on the concentration of ^{99m}Tc(V)-DMSA

Having established that wet labelling is responsible for the formation of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA, I decided to examine the concentration of ^{99m}Tc(V)-DMSA at times after preparation. Samples of ^{99m}Tc-DMSA were prepared by wet labelling at a radioactive concentration of 130 MBq/2.5 ml and by manufacturer's technique at 1.3 GBq/5 ml. These activities were chosen to reflect a typical situation in routine practice in which up to ten patient doses of ^{99m}Tc-DMSA, each containing 130 MBq, are required from a DMSA kit. Analysis by HPLC was performed at 10, 30, 60, 120 and 240 minutes after preparation. Recoveries from the column were measured. To overcome the problem of ^{99m}Tc retained on the column from a previous sample being slowly eluted and interfering with the recovery measurement, each experiment was performed on a separate day. This allowed sufficient time for any ^{99m}Tc retained on the column to decay to an insignificant level. The chromatograms from the 10, 60 and 240 minute analyses are shown in Figures 5.12 and 5.13 To allow comparison between the chromatograms, the counts in each have been corrected for the different radioactive concentrations of the ^{99m}Tc-DMSA prepared by the two labelling techniques and the decay that occurred between preparation and analysis. The recoveries from the column are shown in Table 5.1.

These results demonstrate that with both labelling techniques:

- the ^{99m}Tc(V)-DMSA complexes are formed during the preparation of ^{99m}Tc-DMSA
- the concentration of ^{99m}Tc(V)-DMSA decreases with time after preparation

of the radiopharmaceutical

- the ^{99m}Tc(III)-DMSA complex/complexes are retained on the column
- the activity of ^{99m}Tc retained on the column increases with time after preparation of the radiopharmaceutical

Table 5.1 Results of the comparison of column recoveries of ^{99m}Tc-DMSA prepared by wet labelling and the recommended Amersham technique.

Time after preparation (minutes)	*% recovery from column*	
	wet labelling	*Amersham labelling*
10	45	28
30	33	11
60	23	9
120	14	6
240	10	7

It would appear therefore, that irrespective of the labelling technique, the formation of ^{99m}Tc(V)-DMSA occurs during the preparation of ^{99m}Tc-DMSA and that over a period of time the ^{99m}Tc(V)-DMSA converts to a species that adsorbs on the column. It could be the case that the formation of ^{99m}Tc(V)-DMSA is an intermediate step in the formation of ^{99m}Tc(III)-DMSA but it is impossible to analyse the reaction mixture fast enough to detect a situation in which 100% of the ^{99m}Tc is in the form of ^{99m}Tc(V)-DMSA. Further speculation could lead to the proposal that when ^{99m}Tc(V)-DMSA is prepared as a radiopharmaceutical, the alkaline pH of the reaction mixture prevents the conversion from ^{99m}Tc(V)-DMSA to ^{99m}Tc(III)-DMSA.

While both labelling techniques result in the formation of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA, the manufacturer's technique is superior since the ^{99m}Tc(V)-DMSA falls to an insignificant level within 30 minutes of preparation. As a result of this finding, the routine preparation of ^{99m}Tc(III)-DMSA by the wet labelling technique was discontinued in this radiopharmacy.

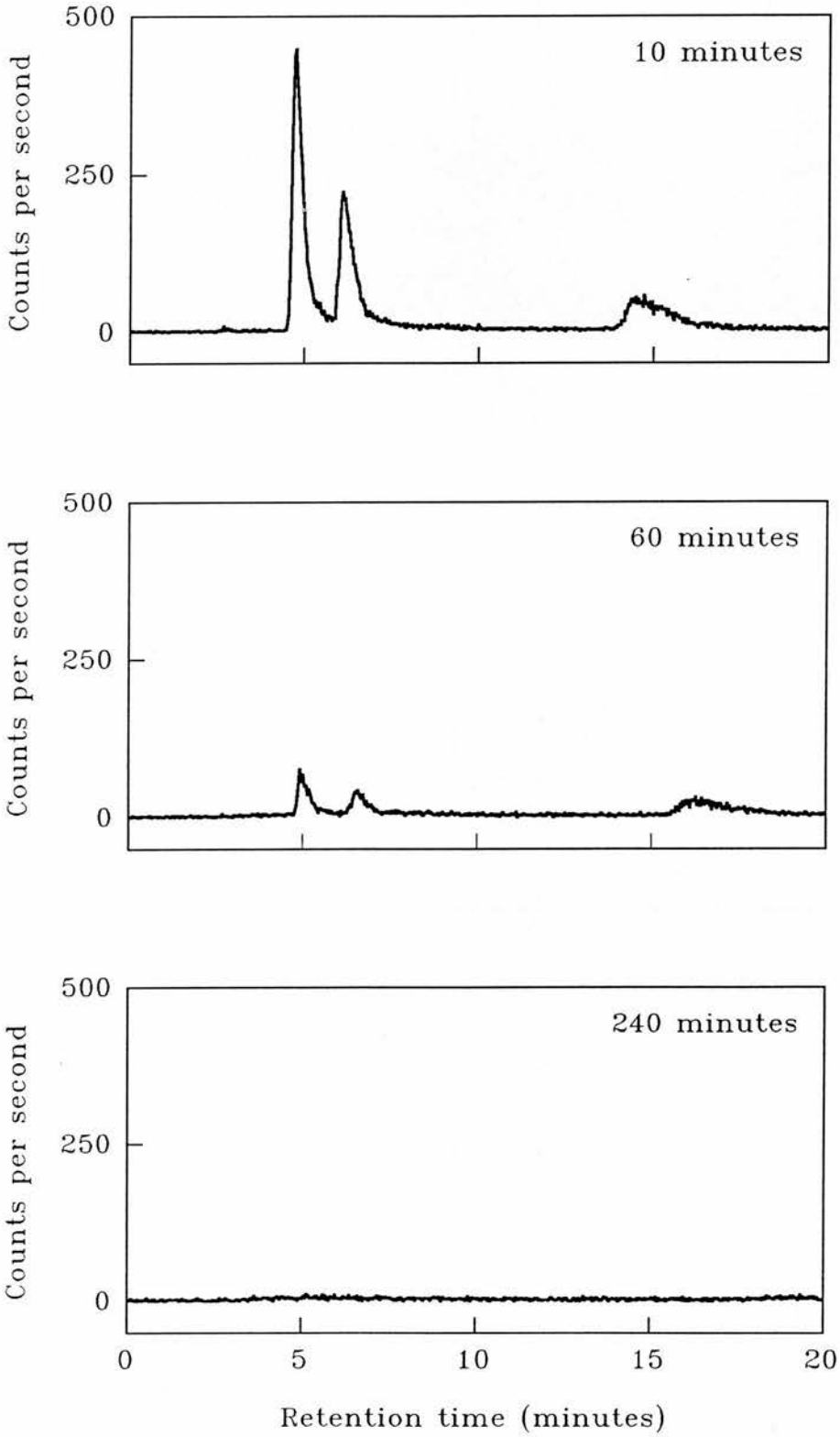

Figure 5.12 *High performance liquid chromatograms of ^{99m}Tc-DMSA at 10, 60 and 240 minutes after preparation by the wet labelling technique.*

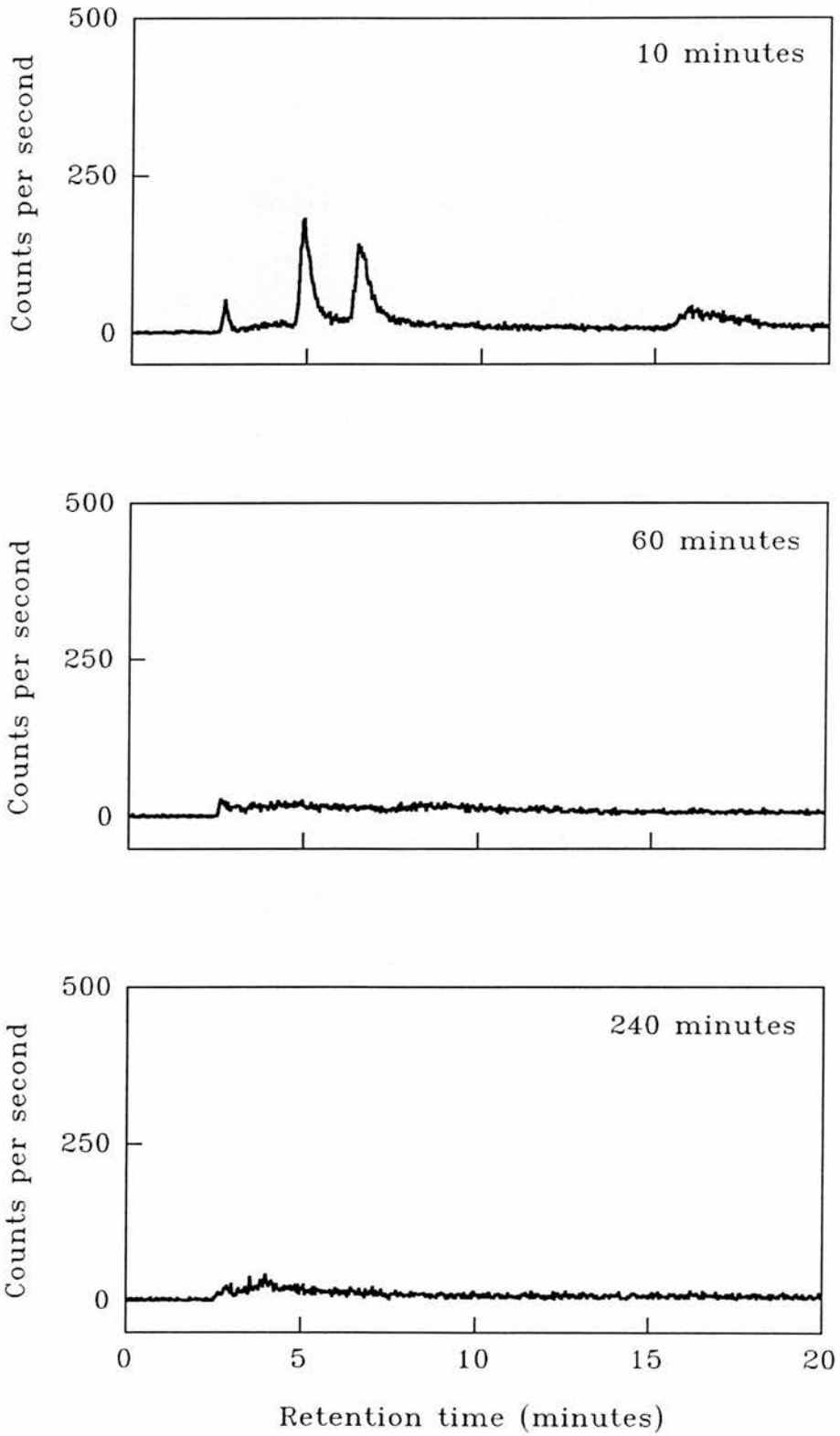

Figure 5.13 *High performance liquid chromatograms of ^{99m}Tc-DMSA at 10, 60 and 240 minutes after preparation by the technique recommended by Amersham.*

5.12 Effect of the presence of ^{99m}Tc(V)-DMSA on urinary excretion

As confirmation of the undesirability of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA, I decided to compare the renal excretion of ^{99m}Tc-DMSA with low and high concentrations of ^{99m}Tc(V)-DMSA. The comparison was carried out in a healthy volunteer (AMM) with no history of kidney disease. Arnold et al. (1975) have shown that approximately 35% of ^{99m}Tc-DMSA administered for kidney imaging appears in the 24 hours after administration. In contrast, Watkinson et al. (1990) have shown that approximately 70% of ^{99m}Tc(V)-DMSA is excreted in the urine in 24 hours. ^{99m}Tc-DMSA that contains a high concentration of ^{99m}Tc(V)-DMSA would therefore be expected to show higher renal excretion than pure ^{99m}Tc(III)-DMSA.

The following methods for preparing the radiopharmaceuticals were designed to give an injection of 125 μg of DMSA. This dose was chosen since Amersham state that up to eight patient doses can be taken from a DMSA kit which contains 1 mg of DMSA. Although 80 MBq is the maximum usual activity recommended by the Administration of Radioactive Substances Committee (1993) for renal imaging, I decided to use only 5 MBq since the measurements were to be undertaken in a volunteer and this activity should provide an adequate count-rate from a sample of urine. The radiation dose from this experiment was an effective dose equivalent of approximately 100 μSv which represents 5% of the national average annual radiation dose from background radiation and 0.2% of the permitted annual whole body dose that can be received by a radiation worker (Ionising Radiations Regulations, 1985). Unfortunately, the low radioactive concentration of the radiopharmaceuticals precluded their simultaneous analysis by HPLC.

To obtain ^{99m}Tc(III)-DMSA containing a high level of ^{99m}Tc(V)-DMSA, the radiopharmaceutical was prepared by the following modification of the wet labelling technique. The kit was reconstituted with 10 ml of Sodium Chloride Injection and 1.6 ml of the solution was transferred to a sterile vial. Sodium Pertechnetate [^{99m}Tc] Injection (6 MBq/2.4 ml) was then injected into the vial. After incubation at room temperature for 10 minutes, 3.2 ml (5 MBq) was drawn into a syringe and the count-rate from the solution was measured with the sodium iodide scintillation detector used in the determination of HPLC column recoveries (Figure 2.4). A second syringe containing a similar activity was prepared for use as a standard. The count-rate from the standard syringe was also measured. The ^{99m}Tc-DMSA was administered intravenously and the count-rate from the residual ^{99m}Tc in the syringe was measured. Urine was collected for 24 hours. The

contents of the standard syringe were diluted to 1 litre and 5 ml was pipetted into a sample tube. The volume of urine was measured and 5 ml was pipetted into a sample tube. The count-rates from the two tubes were measured in an automatic sample counter (Model 1828 Compugamma, LKB Wallac). After correction of all count-rates for background, the percentage of injected ^{99m}Tc in the urine was calculated using the formula:

$$\% \text{ of injected } ^{99m}\text{Tc excreted in urine} = \frac{100\ C_u\ V_u\ C_{ss}}{C_{ds}\ V_s\ C_{is}}$$

where C_u = count-rate from 5 ml of urine

C_{ds} = count-rate from 5 ml of diluted standard

V_u = volume of urine

V_s = volume of diluted standard

C_{ss} = count-rate from standard syringe

C_{is} = count-rate from injected solution i.e. count-rate from syringe containing the injection minus residual count-rate from syringe after injection.

The percentage of the injected ^{99m}Tc that appeared in the urine was found to be 51%.

To obtain the more "pure" ^{99m}Tc(III)-DMSA, the radiopharmaceutical was prepared by reconstituting a DMSA kit with Sodium Pertechnetate [^{99m}Tc] Injection (40 MBq/1.0 ml), incubating the vial at room temperature for 15 minutes then diluting the contents to 6 ml with Sodium Chloride Injection. A syringe containing 0.75 ml of the radiopharmaceutical and a standard syringe were prepared and measured as in the first experiment. The radiopharmaceutical was injected intravenously and the study was performed as described above. The 24 hour urinary excretion was found to be 15%.

As expected, ^{99m}Tc-DMSA containing a high level of ^{99m}Tc(V)-DMSA shows much higher renal excretion than the more pure ^{99m}Tc(III)-DMSA. These results are in line with the value quoted in the literature. The 51% excretion for the radiopharmaceutical containing ^{99m}Tc(V)-DMSA is lower than the 70% quoted by Watkinson (1990) but this can be attributed to some of the ^{99m}Tc being in the form of ^{99m}Tc(III)-DMSA.

This experiment therefore demonstrates that the presence of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA results in higher renal excretion of the ^{99m}Tc. In the routine use of ^{99m}Tc-DMSA

for renal imaging, high excretion could lead to an inaccurate diagnosis of renal pathology. ^{99m}Tc-DMSA's mode of action is to delineate areas of functioning renal parenchyma. Non-functioning tissue appears on the image as areas of reduced or no uptake of ^{99m}Tc. In a situation where there is excretion of ^{99m}Tc into the urine, ^{99m}Tc in the collecting system of the kidney could mask an area of reduced uptake in the renal parenchyma. It would be possible for this situation to arise if ^{99m}Tc-DMSA is heavily contaminated with ^{99m}Tc(V)-DMSA. It is also the case that the presence of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA that is administered for kidney imaging results in less than 100% of the ^{99m}Tc being present in the form that can be concentrated by the kidneys. Uptake will therefore be reduced. If absolute uptake is being used as a measure of kidney function then an erroneously low result will be obtained. The concentration of ^{99m}Tc(V)-DMSA has been shown to vary with time after preparation. Serial measurements of renal function by absolute uptake will therefore be affected and may be meaningless. This demonstration of the effect of ^{99m}Tc(V)-DMSA on renal excretion therefore demonstrates the importance of ^{99m}Tc-DMSA with high radiochemical purity.

5.13 Summary

The work described in this chapter has not resulted in a satisfactory technique for determining the radiochemical purity of ^{99m}Tc-DMSA. However, I am unable to conclude that HPLC as a technique is unsatisfactory for the analysis of ^{99m}Tc-DMSA since I have been unable to investigate the many combinations of stationary and mobile phases that might be used. Chromatographic conditions other than those that I have been able to investigate may not result in adsorption of the ^{99m}Tc(III)-DMSA complex on the column and may be suitable for the analysis of the radiopharmaceutical. Nevertheless, I have demonstrated the following facts, some of which have implications for the routine preparation of ^{99m}Tc-DMSA:

1. The HPLC technique of Moretti et al. (1982) is not satisfactory for determining the radiochemical purity of ^{99m}Tc-DMSA.

2. The levels of ^{99}Tc in ^{99m}Tc encountered in routine radiopharmacy practice have not been shown to influence the radiochemical purity of ^{99m}Tc-DMSA.

3. There is strong adsorption of one or more ^{99m}Tc/DMSA complexes on the HPLC column packing materials ODS, PLRP-S, and Hypercarb.

4. There is adsorption of one or more ^{99m}Tc/DMSA complexes on the stainless-steel sample loop of the HPLC injection valve.

5. ^{99m}Tc(V)-DMSA is a common impurity in ^{99m}Tc-DMSA. The concentration of this impurity decreases with time.

6. The technique specified in the B.P. (1993) for measuring the radiochemical purity of ^{99m}Tc-DMSA is inadequate.

7. Preparation of ^{99m}Tc-DMSA by wet labelling results in a higher concentration of ^{99m}Tc(V)-DMSA than preparation by the manufacturer's technique. This is a consequence of the higher dilution of the DMSA kit during wet labelling.

8. Preparation of ^{99m}Tc-DMSA at the manufacturer's maximum reconstitution volume results in an unacceptably high level of ^{99m}Tc(V)-DMSA at the end of the recommended 10 minute incubation time.

9. The presence of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA results in unacceptably high urinary excretion of ^{99m}Tc.

Although this work has revealed several previously unknown facts about ^{99m}Tc-DMSA, the chromatographic conditions that I investigated have not yielded a satisfactory technique for determining the radiochemical purity of this radiopharmaceutical.

6. Radiochemical Purity of ^{99m}Tc-Exametazime Injection

The work described in this chapter was undertaken to validate an HPLC method for determining the radiochemical purity of ^{99m}Tc-exametazime and then to use the HPLC technique in an investigation of the effects of factors that influence the preparation of this radiopharmaceutical in routine practice.

6.1 Introduction

Technetium-99m [^{99m}Tc] exametazime is a relatively new radiopharmaceutical that is used in tomographic imaging of regional cerebral blood flow (Neirinckx et al. 1987) and for the in-vitro radiolabelling of autologous leucocytes which are used in imaging sites of inflammation (Peters et al. 1986). When used in imaging of regional cerebral blood flow, ^{99m}Tc-exametazime is injected intravenously into the patient and, being a low molecular weight lipophilic molecule with zero charge, crosses the blood/brain barrier. Following its transit of the barrier, ^{99m}Tc-exametazime interacts with an intracerebral biomolecule, possibly glutathione, and undergoes a molecular change which prevent its egress from the brain (Neirinckx et al. 1988). The ^{99m}Tc therefore becomes trapped inside the brain and allows imaging to be performed.

For the detection of sites of inflammation by radionuclide imaging, leucocytes are first isolated from a sample of the patient's blood. The leucocytes are then incubated with ^{99m}Tc-exametazime which is taken up by the cells and selectively retained by the neutrophils. After washing to remove the ^{99m}Tc that has not been retained in cells, the radiolabelled leucocytes are reinjected into the patient. Upon reinjection, the ^{99m}Tc-labelled neutrophils behave normally and become concentrated in sites of inflammation which can then be detected by imaging with a gamma-camera.

^{99m}Tc-exametazime is prepared using Ceretec, a commercially available radiopharmaceutical kit that is manufactured by Amersham International. The Ceretec kit consists of a vial which contains a sterile freeze-dried mixture of 0.5 mg exametazime ([RR,SS]-4,8-diaza-3,6,6,9-tetramethyl undecane-2,10-dione bisoxime), 7.6 μg stannous chloride dihydrate and 4.5 mg sodium chloride. The vial contains a nitrogen atmosphere to prevent oxidation of the stannous chloride. ^{99m}Tc-exametazime is prepared by injecting Sodium Pertechnetate [^{99m}Tc] Injection into a Ceretec kit. The pH of the resulting

intravenous injection is in the range 9.0 - 9.8. Upon addition of ^{99m}Tc-pertechnetate to the kit, the ^{99m}Tc undergoes reduction by the stannous ion and, in the reduced state, forms a complex with the exametazime. The structure of the complex is shown in Figure 6.1 (Nowotnik et al. 1985).

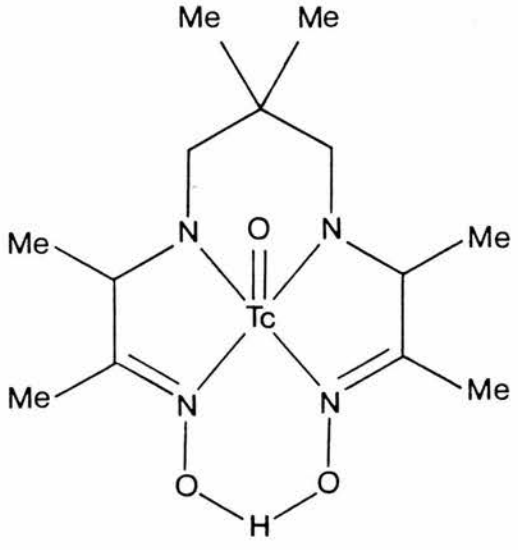

Figure 6.1 *Proposed structure of ^{99m}Tc-exametazime*

Amersham International (1989) recommends that the following conditions are observed when reconstituting a Ceretec kit:

1. The sodium pertechnetate [^{99m}Tc] that is used to reconstitute the kit must have been eluted from a ^{99m}Tc generator less than two hours previously. The rationale for this restriction is the build-up of free radicals that are formed as a result of the energy dissipated in radioactive solutions (Bayne et al. 1989). If present in a solution that is used to prepare ^{99m}Tc-exametazime, these species can cause oxidation of the complex which results in the release of ^{99m}Tc in the form of ^{99m}Tc-pertechnetate impurity.

2. The previous elution of the ^{99m}Tc generator must have been within the preceding 24 hours. The longer that a generator remains uneluted, the greater is the build-up of ^{99}Tc and the higher is the ^{99}Tc/^{99m}Tc ratio in the

eluate when elution is eventually performed. ^{99}Tc is of no value since it does not emit gamma radiation and, in a labelling reaction, ^{99}Tc and ^{99m}Tc are indistinguishable. The restriction on the time since the previous elution is related to the total amount of Tc, i.e. ^{99}Tc and ^{99m}Tc, that can be accommodated by the Ceretec kit. The ^{99m}Tc-exametazime complex is formed at a pH of 9.0 - 9.8. In this pH range, the stannous salts that are used as reducing agents in ^{99m}Tc radiopharmaceuticals are relatively insoluble. The concentration of stannous chloride in Ceretec is therefore much lower than in other ^{99m}Tc radiopharmaceutical kits. As a result of this lower than normal reducing capacity, the amount of Tc that is added to a Ceretec kit must be carefully controlled otherwise there will be insufficient stannous ion to reduce all the Tc and a product containing unlabelled Tc will be produced.

3. Not more than 1.1 GBq of ^{99m}Tc must be added to the kit. The purpose of this restriction is also to control the amount of Tc that is added to the kit.

4. Administration of the ^{99m}Tc-exametazime to the patient must be within 30 minutes of reconstitution of the Ceretec kit. This time limit is a consequence of the formation of impurities which reduce the radiochemical purity of the radiopharmaceutical. The lipophilic ^{99m}Tc-exametazime complex, commonly referred to as primary ^{99m}Tc-exametazime complex, undergoes conversion to a less lipophilic species, referred to as secondary ^{99m}Tc-exametazime complex (Neirinckx et al. 1987). This complex does not cross the blood/brain barrier and is therefore a radiochemical impurity. ^{99m}Tc-exametazime also undergoes oxidation with the release of ^{99m}Tc in the form of ^{99m}Tc-pertechnetate impurity. The rates at which these impurities form are such that satisfactory radiochemical purity can only be guaranteed up to 30 minutes after reconstitution of the Ceretec kit.

In addition to those described above, unbound reduced ^{99m}Tc has also been reported as a radiochemical impurity that can be present in ^{99m}Tc-exametazime (Neirinckx et al. 1987).

To be suitable for determination of the radiochemical purity of ^{99m}Tc-exametazime, an analytical technique should be specific for the primary ^{99m}Tc-exametazime complex and be capable of providing a measure of the level of this species. The standard procedure

recommended by the manufacturer of Ceretec involves the use of a combination of thin-layer and paper chromatography (Amersham 1989). This procedure suffers from the disadvantages that it is not specific for the primary ^{99m}Tc-exametazime complex and the paper chromatogram takes 100 minutes to run the 15 cm recommended. For a radiopharmaceutical with a useful life of only 30 minutes, this time seems inappropriate as a result cannot be made available before the ^{99m}Tc-exametazime is injected into the patient. Also, the long analysis time does not facilitate investigation of the radiopharmaceutical over its 30 minute life.

Various attempts have been made to develop more rapid analytical techniques. Solanki et al. (1988) investigated the use of paper chromatography on Whatman DE 81, a weakly basic ion exchange cellulose, with butan-2-one as the solvent. In this system, primary ^{99m}Tc-exametazime complex migrates with the solvent front while the radiochemical impurities remain on the origin. No comparison with any other technique was reported. The use of paper chromatography on Whatman No.1 with diethyl ether as the solvent was reported by Hung et al. (1988). As with the previous system, primary ^{99m}Tc-exametazime complex travels with the solvent front while the impurities remain at the origin. The results obtained with this technique were compared with results from two other techniques. Results were in good agreement with those from the standard Amersham thin-layer/paper technique but in poor agreement with an HPLC technique which was reported to under-estimate the percentage of ^{99m}Tc-pertechnetate impurity and thereby result in an over-estimate of primary ^{99m}Tc-exametazime complex. Ballinger et al. (1988) investigated an extraction technique in which a sample of ^{99m}Tc-exametazime is mixed with ethyl acetate. Primary ^{99m}Tc-exametazime complex is extracted into the organic phase while the radiochemical impurities remain in the aqueous phase. Results from this technique were shown to agree well with results obtained with the standard Amersham thin-layer/paper chromatography technique. The use of a disposable pre-packed reversed phase chromatography cartridge (Sep-Pak C18, Waters Associates) was investigated by Mah et al. (1989). With this technique, primary ^{99m}Tc-exametazime complex is retained in the cartridge while the impurities are washed through with the 0.9% sodium chloride eluent. Results obtained with this technique were found to agree reasonably well with those from the standard Amersham technique but poorly with those from the Ballinger solvent extraction technique.

As discussed in Chapter 1, each of the above techniques can be criticised for their lack of specificity for the primary ^{99m}Tc-exametazime complex. The thin-layer and paper

chromatography techniques rely on the various species in the radiopharmaceutical either migrating with the solvent front or being retained at the origin. The other two techniques also rely upon a similar "origin/solvent front" approach. In the extraction technique, the various species either remain in the aqueous phase or partition into an organic phase while in the cartridge technique the species are either retained in or eluted from the cartridge. Although these techniques appear to cope reasonably satisfactorily with the well recognised impurities in ^{99m}Tc-exametazime, it is doubtful if they would be adequate for detecting an unusual impurity which might behave in an identical manner to primary ^{99m}Tc-exametazime complex.

An HPLC technique that produces a chromatogram containing a peak which is characteristic of the primary ^{99m}Tc-exametazime complex should therefore constitute a more specific technique for determination of radiochemical purity.

During the development of ^{99m}Tc-exametazime, HPLC was used to study the various species present in the radiopharmaceutical (Neirinckx et al. 1987). However, the HPLC technique was not used to determine radiochemical purity. This was performed using the thin-layer/paper chromatography technique that was subsequently adopted as the standard Amersham technique. No comparison of results from the HPLC and thin-layer/paper chromatography techniques was presented.

The work documented in this chapter was therefore undertaken to:

1. develop and validate an HPLC technique for measuring the radiochemical purity of ^{99m}Tc-exametazime

2. use the HPLC technique to develop a method that can be used routinely to stabilize ^{99m}Tc-pertechnetate that is supplied for the preparation of ^{99m}Tc-exametazime

3. use the HPLC technique to establish if the radiochemical purity and stability of ^{99m}Tc-exametazime is influenced by the source and age of the ^{99m}Tc-pertechnetate used in its preparation

6.2 Comparison of HPLC and thin-layer/paper chromatography

The HPLC technique that I chose to investigate was based on the method described by Neirinckx et al. (1987). The equipment used was the Philips PU4100 pump, the Rheodyne 7125 valve fitted with a 20 μl sample loop, the radiation detector in configuration A (Figure 2.1), the Specmate pre-amplifier/amplifier/high voltage supply and the Accuspec multichannel analyser. Separation was carried out using gradient elution on a 150 x 4.6 mm PRP-1 column (Hamilton) which was fitted with a 25 x 3 mm guard column. The column was eluted with 20 mM phosphate buffer pH 7.4 at a flow-rate of 2 ml/min. Immediately after injection of a 20 μl sample of the radiopharmaceutical, tetrahydrofuran was introduced into the mobile phase in a linear gradient of 0-25% over 6 minutes. The buffer/tetrahydrofuran mixture (75:25) was maintained for 4 minutes then the tetrahydrofuran was withdrawn from the mobile phase in a linear gradient 25-0% over 6 minutes. Phosphate buffer was then pumped through the column for a further 12 minutes before the column was used to perform another analysis. Acquisition of the chromatogram and measurement of recovery from the column were performed according to the techniques described in Chapter 3. A typical chromatogram is shown in Figure 6.2.

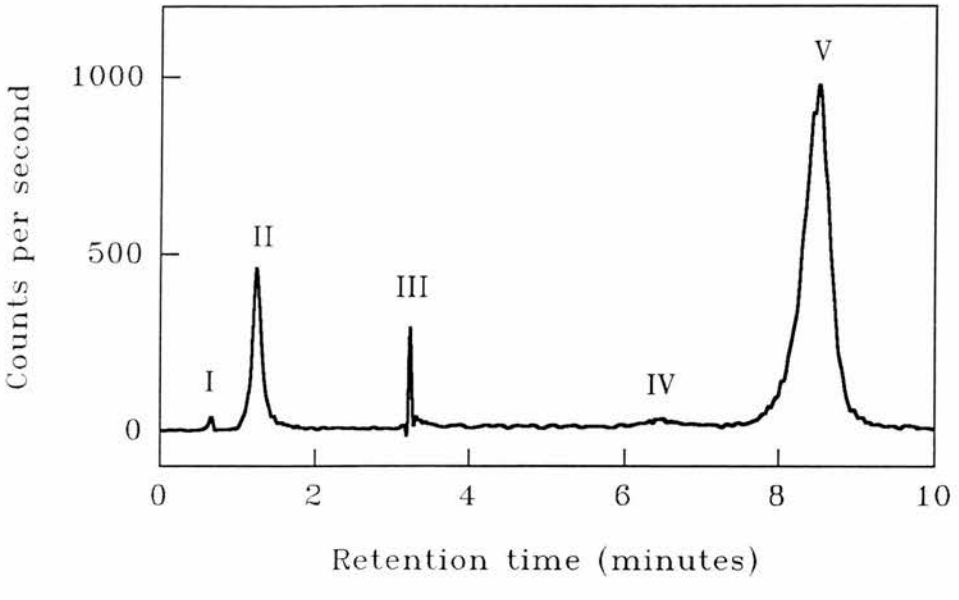

Figure 6.2 *High performance liquid chromatogram of ^{99m}Tc-exametazime.*

6. Radiochemical Purity of ^{99m}Tc-exametazime Injection

The chromatogram contains the following four peaks:

I	an unidentified impurity
II	^{99m}Tc-pertechnetate impurity
III	secondary ^{99m}Tc-exametazime
IV	an unidentified impurity
V	primary ^{99m}Tc-exametazime

A region of interest was placed around each peak and the counts in each region were recorded and corrected for background. The counts in each peak were expressed as a percentage of the total counts in the chromatogram. The percentage in the peak corresponding to primary ^{99m}Tc-exametazime complex was taken to be the radiochemical purity.

The thin-layer/paper chromatographic analysis was performed according to the standard Amersham technique. This employs a combination of three chromatographic systems as follows:

System 1: An ascending paper chromatography tank (Shandon) was filled to a depth of 1 cm with fresh acetonitrile/water (50:50) and allowed to equilibrate for one hour. A 2.5 x 30 cm strip was cut from a 46 x 57 cm sheet of Whatman No.1 chromatography paper. The machine direction was marked to ensure that the solvent was run in this direction. The origin was marked 2.5 cm from the bottom of the strip. The solvent front was marked 10 cm above the origin. One drop of the ^{99m}Tc-exametazime to be analysed was applied to the origin using a 1 ml hypodermic syringe fitted with a 25G needle. The strip was placed immediately in the chromatography tank. The time taken for the solvent to travel the 10 cm to the solvent front was approximately 60 minutes. When the solvent front was reached, the strip was removed from the tank and dried with a hot air blower. The strip was then cut transversely into 11 x 1 cm sections, starting 5 mm below the origin. The first section therefore contained the origin and the last section contained the solvent front. The sections were placed in sample tubes and the count-rate from each was determined using an automatic gamma sample counter (Model 1282 Compugamma, LKB Wallac). The counting time was chosen to ensure that at least 10,000 counts were recorded from the most active section. With this system, unbound reduced ^{99m}Tc remains at the origin while primary ^{99m}Tc-exametazime, secondary ^{99m}Tc-exametazime and ^{99m}Tc-pertechnetate impurity migrate with an Rf of 0.8-1.0. Using the general procedure

described in Chapter 3 for the analysis of chromatograms, the level of unbound reduced ^{99m}Tc (R%) in the radiopharmaceutical was calculated by expressing the counts at the origin as a percentage of the total counts on the strip.

System 2: An ascending thin-layer chromatography tank (Gelman) was filled to a depth of 1 cm with a fresh solution of 0.9% sodium chloride and allowed to equilibrate for one hour. A 2.5 x 20 cm plate was cut from a 20 x 20 cm sheet of Instant Layer Chromatography/Silica Gel (ITLC/SG, Gelman). The origin was marked 2.5 cm from the bottom of the plate. The solvent front was marked 10 cm above the origin. The sample was applied as described for System 1 and the plate was placed immediately in the chromatography tank. The plate was developed, dried, cut and counted as described above. With this system, unbound reduced ^{99m}Tc, primary ^{99m}Tc-exametazime and secondary ^{99m}Tc-exametazime remain at the origin while ^{99m}Tc-pertechnetate impurity migrates with an Rf of 0.8-1.0. The level of ^{99m}Tc-pertechnetate impurity (P%) in the radiopharmaceutical was therefore calculated by expressing the counts at the solvent front as a percentage of the total counts on the plate.

System 3: This procedure was identical to System 2 except that a solvent of butan-2-one was used. With this system, unbound reduced ^{99m}Tc and secondary ^{99m}Tc-exametazime complex remain at the origin while ^{99m}Tc-pertechnetate impurity and primary ^{99m}Tc-exametazime migrate with an Rf of 0.8-1.0. The combined level of unbound reduced ^{99m}Tc and secondary ^{99m}Tc-exametazime was therefore calculated by expressing the counts at the origin as a percentage of the total counts on the plate. The level of secondary ^{99m}Tc-exametazime (2°%) was then calculated by subtracting the level of unbound reduced ^{99m}Tc (R%) determined with System 1 from the combined level determined with System 3.

The level of primary ^{99m}Tc-exametazime complex (1°%) was then calculated as:

$$1°\% = 100 - (P\% + R\% + 2°\%)$$

The level of primary ^{99m}Tc-exametazime complex was taken as the measure of radiochemical purity.

Each of the above chromatograms was run over a distance of only 10 cm rather than the 15 cm recommended by Amersham. The shorter distance was used due to the unacceptably long 100 minutes required for the Whatman No.1/50% acetonitrile system to run the

recommended 15 cm. A typical set of chromatograms is shown in Figure 6.3. For each system, the 10 cm distance provides adequate separation of the species in ^{99m}Tc-exametazime.

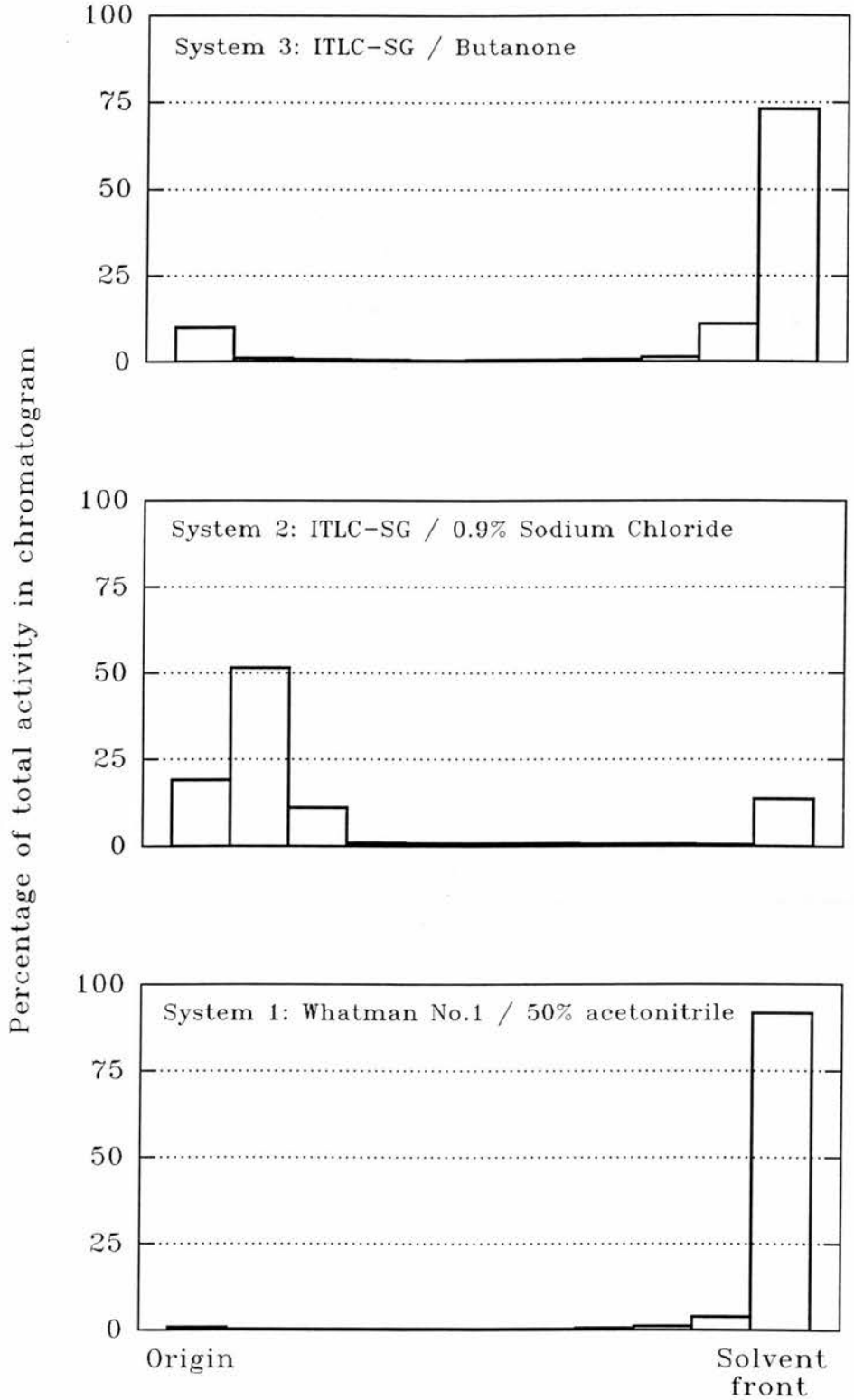

Figure 6.3 *Thin-layer and paper chromatograms of ^{99m}Tc-exametazime.*

6. Radiochemical Purity of ^{99m}Tc-exametazime Injection

To compare the results obtained with HPLC and thin-layer/paper chromatography, I used both techniques to analyse 24 samples of ^{99m}Tc-exametazime with a wide range of radiochemical purities (54.6-94.5%). The mean level ± standard deviation of each species detected is shown in Table 6.1. The mean ± standard deviation radiochemical purity determined by the thin-layer/paper technique was 81.5 ± 10.5% and by the HPLC technique was 81.2 ± 10.2%. When compared using a paired Student's t-test, these results were not found to be significantly different ($P > 0.30$). The excellent correlation ($r = 0.98$) between the two techniques is shown in Figure 6.4.

Table 6.1 Results of the comparison of High Performance Liquid Chromatography (HPLC) and Thin-Layer/Paper Chromatography (TLC/PC) for the analysis of ^{99m}Tc-exametazime.

Species	*% of total activity*	
	HPLC	*TLC/PC*
Unknown 1	0.3 ± 0.3%	-
^{99m}Tc-pertechnetate	6.8 ± 7.9	6.5 ± 8.7%
2° ^{99m}Tc-exametazime	7.1 ± 3.8	10.4 ± 4.0
Unknown 2	4.6 ± 0.7	-
1° ^{99m}Tc-exametazime	81.2 ± 10.2	81.5 ± 10.5
Recovery	98.9 ± 1.1	-

Each value is the mean ± standard deviation of five results

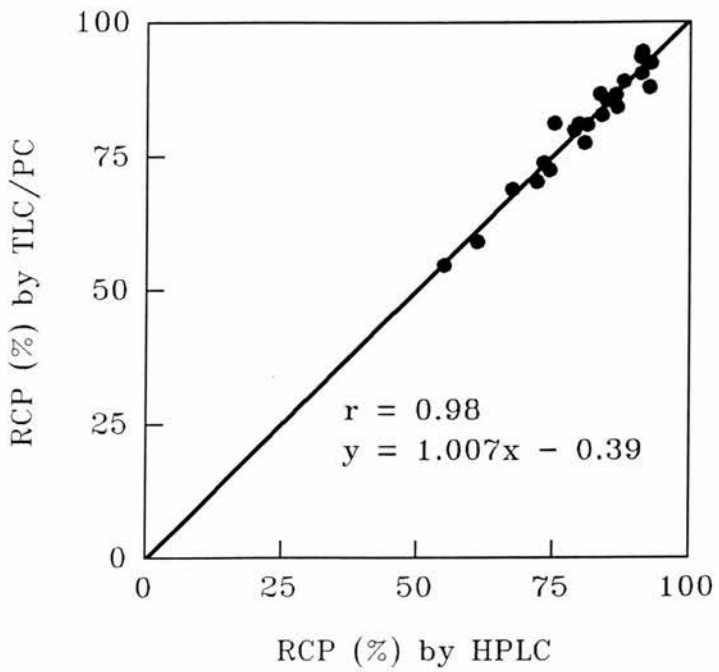

Figure 6.4 *Comparison of the radiochemical purity (RCP) of ^{99m}Tc-exametazime as measured by HPLC and TLC/PC.*

Hung et al. (1988) reported that the HPLC technique used in their study resulted in an under-estimate of ^{99m}Tc-pertechnetate impurity when compared to the ITLC/SG:0.9% sodium chloride system of the Amersham thin-layer/paper chromatographic technique. The results of the above study do not corroborate this finding in that the levels of ^{99m}Tc-impurity measured by the two techniques do not differ significantly.

From the results of my study, it can be concluded that over the range of radiochemical purities likely to be encountered in practice, the HPLC technique investigated in this work is satisfactory for determination of the radiochemical purity of ^{99m}Tc-exametazime.

6.3 Use of sodium iodide to stabilize sodium pertechnetate [^{99m}Tc] dispensed for the preparation of ^{99m}Tc-exametazime

A major limitation in the use of ^{99m}Tc-exametazime is the expiry time of 30 minutes after reconstitution of the Ceretec kit. A consequence of this short expiry is that the radiopharmaceutical must be prepared immediately before it is to be used. This may mean that reconstitution of the kit cannot be undertaken in the radiopharmacy but must be carried out in the department in which the ^{99m}Tc-exametazime is to be used. Amersham also recommend that the kit is reconstituted with sodium pertechnetate [^{99m}Tc] obtained from a generator eluate that is less than 2 hours old. This can represent a logistical problem if the nuclear medicine department is in a different institution to the radiopharmacy or if the ^{99m}Tc-exametazime is to be used more than 2 hours after the time at which the ^{99m}Tc generator is eluted routinely. Bayne et al. (1989) have reported that the addition of sodium iodide to the generator eluate overcomes this problem and extends the shelf-life of sodium pertechnetate [^{99m}Tc] from 2 to 6 hours. I have therefore undertaken the development of a method by which this procedure can be introduced into routine use. In the course of this work, the HPLC technique that has been validated for the analysis of ^{99m}Tc-exametazime was used to assess the stability of ^{99m}Tc-exametazime.

As a convenient means of using the stabilization technique routinely, I decided to investigate a kit approach by preparing batches of vials containing sodium iodide. The principle behind this technique was that on each occasion that sodium pertechnetate [^{99m}Tc] is required for the preparation of ^{99m}Tc-exametazime, it will be dispensed into a kit within 30 minutes of generator elution.

6.3.1 Preparation of sodium iodide kits

Most commercially manufactured radiopharmaceutical kits are formulated as freeze-dried products. Since freeze-drying is not a technique available to most hospital radiopharmacies, I decided to investigate the suitability of a kit containing a solution of sodium iodide. Bayne et al. (1989) demonstrated that 400 μg of sodium iodide was adequate for stabilization of the maximum activity (1.11 GBq) of ^{99m}Tc recommended for the reconstitution of a Ceretec kit. I therefore decided to prepare kits containing 440 μg of sodium iodide in 1.0 ml of Sodium Chloride Injection B.P. The principle behind this technique is as follows. ^{99m}Tc generator eluate, diluted to have a radioactive concentration

of 1.2 GBq in 4.5 ml at the time when the Ceretec kit will be reconstituted, is injected into a sodium iodide kit. The kit then contains 1.2 GBq of ^{99m}Tc and 440 μg of sodium iodide in 5.5 ml. Amersham recommend that the Ceretec kit is reconstituted to a volume of 5 ml. Using 5.0 ml of the ^{99m}Tc/sodium iodide solution prepared as described above results in the Ceretec kit being reconstituted with 1.1 GBq of ^{99m}Tc containing 400 μg of sodium iodide.

Aqueous solution of sodium iodide gradually become coloured on exposure to light and air due to the liberation of iodine (Martindale 1989). I therefore decided to use vials containing a nitrogen atmosphere for preparation of the kits which would then be protected from light. Sodium Chloride Injection B.P. which has a slightly acidic pH is not the ideal solvent for sodium iodide which is most stable under the slightly alkaline conditions of pH 8-9.5 (Merck Index 1976). However, pH adjustment of the solution used to prepare the kits was considered inadvisable since pH is known to be important in the labelling of exametazime. Reconstitution with a pH-adjusted solution might therefore upset the labelling reaction and result in low radiochemical purity.

Sodium Iodide B.P. was therefore dissolved in Sodium Chloride Injection B.P. to give a solution of concentration 440 μg/ml. Kits were prepared by filtering 1.0 ml aliquots of this solution through a sterile 220 nm filter into weighed nitrogen-filled vials (Product Code N46, Amersham). Each vial was reweighed and the weight of solution calculated. From these weights, the sodium iodide content of each kit was calculated. Five batches of kits were prepared. From each batch, 10 vials were stored at room temperature, 10 in a refrigerator at 4°C and 10 in a freezer at -22°C.

6.3.2 Assay of sodium iodide kits

In view of the potential instability discussed above, the first step in determining the feasibility of the sodium iodide kit concept was to measure the stability of the kits and determine if it is influenced by the conditions under which they are stored. To measure stability, I devised a modification of the 1968 B.P. method for assaying the iodide content of sodium iodide. The modification was necessary due to the small volume of solution in each kit and the small amount of sodium iodide present. The 1968 method was preferred to the methods introduced in more recent editions of the B.P. since it was more readily miniaturized. The analytical technique depends upon hydrochloric acid converting iodide to iodine which is then titrated with potassium iodate solution. The presence of iodine in

the kit as a result of oxidation of iodide during storage would not be revealed by this titrimetric analysis. A separate test for the detection of iodine was therefore necessary.

At 0, 1, 4 and 8 weeks after preparation, two kits that had been kept under each storage condition were used in the modified assay to determine iodide content as follows. One vial from each pair was centrifuged to bring all the solution to the bottom of the vial. The rubber cap was removed from the vial. Hydrochloric acid, 1.2 ml, was pipetted into the vial which was then reweighed. Approximately 0.1 ml of 3 mM potassium iodate solution was added to the vial to develop a yellow colour and the vial was reweighed. Amaranth solution (1/100th strength), 0.1 ml, was added to the vial which was reweighed. Potassium iodate solution, 3 mM, was added until the colour of the solution changed from pink to pale yellow. The vial was reweighed. From the total weight of 3 mM potassium iodate solution added, the iodide content of the vial was calculated (each ml of 3 mM potassium iodate solution is equivalent to 899 μg of sodium iodide). One drop of Starch Mucilage B.P. was injected into the other vial of the pair. A blue colour developed if $>1\%$ of the iodine was present in the from of I_2. The results of the iodide assays are shown in Table 6.2

Table 6.2 Effect of time on the iodide content of sodium iodide kits stored under different conditions

Time after preparation (weeks)	*% of declared iodide*		
	room temperature	*4°C*	*-22°C*
0	98.6 ± 0.8%	-	-
1	98.8 ± 1.2	99.5 ± 0.5%	98.5 ± 1.8%
4	98.7 ± 0.9	99.0 ± 0.9	97.9 ± 1.9
8	96.9 ± 0.4	98.2 ± 2.0	98.5 ± 2.3

Each value is the mean ± standard deviation of five results

6. Radiochemical Purity of ^{99m}Tc-exametazime Injection

For each storage condition, the iodide content of the kits remained >96% throughout the 8 weeks of the study. No kit was found to contain >1% of the iodine in the form of I_2. As the method of preparing the kits involves aseptic manipulations which always carry the risk of bacterial contamination, and none of the temperatures tested affect stability, I chose refrigeration as the storage condition. This is convenient in routine practice and will inhibit the growth of any contamination introduced during preparation. I also decided to err on the side of safety and give the kits an expiry date of four weeks after preparation.

6.3.3 Effect of sodium iodide kits on the stability of ^{99m}Tc-exametazime

Having established the stability of the sodium iodide kits, the following experiments were performed to demonstrate their effectiveness in extending the useful life of sodium pertechnetate [^{99m}Tc] that is to be used in the preparation of ^{99m}Tc-exametazime. With a four week expiry having been assigned to the sodium iodide kits, I decided that the most challenging test of their effectiveness would be to use them at expiry.

^{99m}Tc was obtained from 30 GBq ^{99m}Tc generators (Product Code MCC20, Amersham) that had been eluted previously within 24 hours. The generator eluates were diluted immediately after elution. ^{99m}Tc-exametazime injections (1.1 GBq/5 ml) were prepared according to the following three techniques:

1. ***1 h sodium pertechnetate [^{99m}Tc]:*** The volume of generator eluate that contained 1.3 GBq of ^{99m}Tc was diluted to 5.5 ml with Sodium Chloride Injection B.P. and injected into a sterile 10 ml nitrogen-filled vial (Product Code N46, Amersham). One hour later, 5.0 ml of this solution was injected into a Ceretec kit.

2. ***6 h sodium pertechnetate [^{99m}Tc]:*** The volume of generator eluate that contained 2.4 GBq of ^{99m}Tc was diluted to 5.5 ml with Sodium Chloride Injection B.P. and injected into a sterile 10 ml nitrogen-filled vial. Six hours later, 5 ml of this solution was injected into a Ceretec kit.

3. ***6 h sodium pertechnetate [^{99m}Tc] + NaI:*** The volume of generator eluate that contained 2.4 GBq of ^{99m}Tc was diluted to 4.5 ml with Sodium Chloride Injection B.P. and injected into a four week-old sodium iodide

kit, giving a total volume of 5.5 ml. Six hours later, 5 ml of this solution was injected into a Ceretec kit.

Five ^{99m}Tc-exametazime injections were prepared by each of the three techniques. A different batch of Ceretec kits was used for each set of three experiments. The ^{99m}Tc-exametazime injections were stored at room temperature.

The radiochemical purity of each ^{99m}Tc-exametazime injection was measured using the validated HPLC technique. Although the recommended expiry time for ^{99m}Tc-exametazime is 30 minutes, measurements were made at 2, 30, 60 and 120 minutes after preparation to ensure that any tendency towards reduced stability would be clearly demonstrated. The results are shown in Table 6.3. The changes in the levels of the two principal impurities (^{99m}Tc-pertechnetate and secondary ^{99m}Tc-exametazime complex), with time are shown in Figure 6.5

The very high recovery of ^{99m}Tc from the HPLC column (overall mean = 99.8$\pm$2.0%, n = 60) demonstrates that no significant radiochemical impurities in the ^{99m}Tc-exametazime became adsorbed onto the column packing material and thereby remained undetected.

Table 6.3 Effect of three preparations of sodium pertechnetate [^{99m}Tc] on the composition of ^{99m}Tc-exametazime

Sodium pertechnetate [^{99m}Tc]	*Species (peak no.)*	*% of total activity at times after preparation*			
		2 min	*30 min*	*60 min*	*120 min*
1 hour-old	Unknown 1 (I)	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
	^{99m}Tc-pertech (II)	1.2 ± 0.2	1.9 ± 0.9	3.0 ± 2.4	7.1 ± 5.0
	2° ^{99m}Tc-examet (III)	2.6 ± 0.3	4.7 ± 1.4	6.5 ± 3.2	8.8 ± 5.2
	Unknown 2 (IV)	3.3 ± 0.1	3.6 ± 1.4	4.4 ± 1.5	4.5 ± 1.4
	1° ^{99m}Tc-examet (V)	92.8 ± 1.3	89.7 ± 2.4	85.9 ± 4.1	79.2 ± 5.0
	Recovery	99.7 ± 0.3	100.1 ± 1.2	98.3 ± 1.9	99.6 ± 1.7
6 hour-old	Unknown 1 (I)	0.1 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	1.1 ± 0.2
	^{99m}Tc-pertech (II)	1.6 ± 1.3	10.2 ± 2.4	18.0 ± 3.1	30.0 ± 3.8
	2° ^{99m}Tc-examet (III)	2.9 ± 0.5	6.2 ± 0.9	8.7 ± 1.2	10.1 ± 1.6
	Unknown 2 (IV)	2.9 ± 1.3	3.1 ± 1.1	3.3 ± 1.1	2.8 ± 0.9
	1° ^{99m}Tc-examet (V)	92.5 ± 1.7	80.1 ± 2.0	69.3 ± 3.2	56.0 ± 4.0
	Recovery	99.4 ± 1.1	100.2 ± 2.5	98.9 ± 3.0	99.4 ± 2.1
6 hour-old + sodium iodide	Unknown 1 (I)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.0
	^{99m}Tc-pertech (II)	1.0 ± 0.2	1.6 ± 0.1	3.2 ± 0.6	7.2 ± 2.7
	2° ^{99m}Tc-examet (III)	2.5 ± 0.6	5.3 ± 1.4	6.7 ± 2.3	7.8 ± 2.5
	Unknown 2 (IV)	3.2 ± 1.4	3.7 ± 1.7	3.8 ± 1.7	3.5 ± 1.5
	1° ^{99m}Tc-examet (V)	93.2 ± 2.0	89.3 ± 3.0	86.2 ± 4.6	81.2 ± 6.4
	Recovery	100.6 ± 2.3	100.4 ± 2.2	100.5 ± 2.6	101.1 ± 3.3

Each value is the mean ± standard deviation of five results

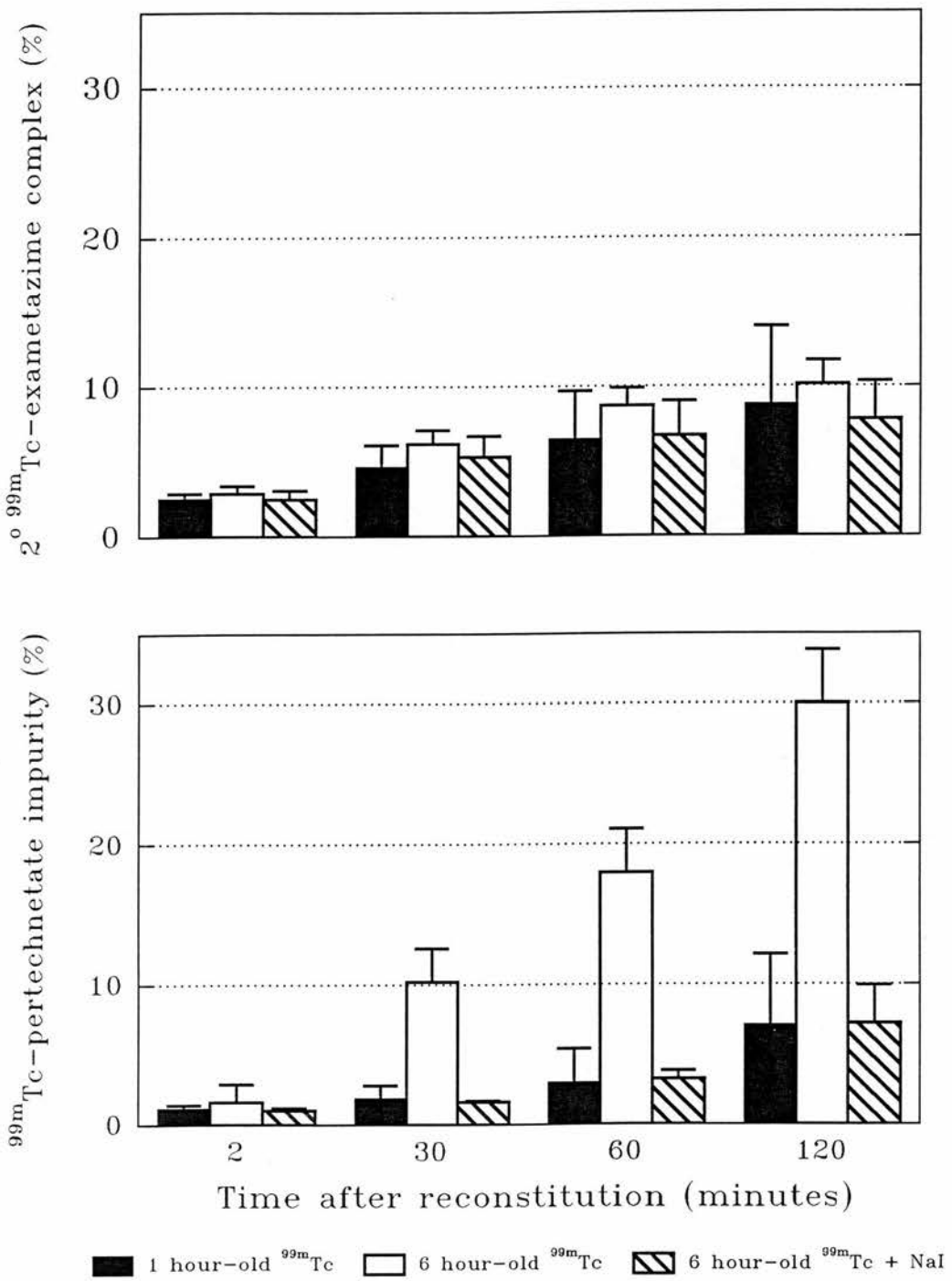

Figure 6.5 *Changes in the levels of secondary ^{99m}Tc-exametazime complex and ^{99m}Tc-pertechnetate impurity in ^{99m}Tc-exametazime.*

6. Radiochemical Purity of ^{99m}Tc-exametazime Injection

Over the 120 minutes of the study, the level of Unknown 1 did not change in the ^{99m}Tc-exametazime prepared with 1 hour-old sodium pertechnetate [^{99m}Tc] or 6 hour-old sodium pertechnetate [^{99m}Tc] + sodium iodide. It did rise significantly when 6 hour-old sodium pertechnetate [^{99m}Tc] was used but at 30 minutes, i.e. at the expiry time of ^{99m}Tc-exametazime, was only 0.4% and does not therefore represent a major impurity.

The level of Unknown 2 did not change in any of the preparations over the course of the study and at approximately 3.5%, also does not represent a major impurity.

Over 120 minutes, the level of secondary ^{99m}Tc-exametazime complex rose steadily and equally in each of the three preparations. Sodium iodide has therefore no effect on the conversion of primary to secondary ^{99m}Tc-exametazime complex.

^{99m}Tc-pertechnetate impurity rose in ^{99m}Tc-exametazime prepared by each of the three techniques. However, the rise was much more rapid in ^{99m}Tc-exametazime prepared using 6 hour-old sodium pertechnetate [^{99m}Tc] than in the other two. The rise in ^{99m}Tc-pertechnetate impurity is therefore the cause of the reduced radiochemical purity that is seen when ^{99m}Tc-exametazime is prepared using 6 hour-old sodium pertechnetate [^{99m}Tc].

The radiochemical purity of ^{99m}Tc-exametazime prepared according to each of the three techniques decreased over the 120 minutes of the study. Technique 1 (1 hour-old sodium pertechnetate [^{99m}Tc]) meets all the conditions prescribed by Amersham and was therefore be assumed to yield "standard" ^{99m}Tc-exametazime. The radiochemical purities obtained with 6 hour-old sodium pertechnetate [^{99m}Tc] (technique 2) and 6 hour-old sodium pertechnetate [^{99m}Tc] + sodium iodide (technique 3) were therefore compared to those obtained with technique 1. Since the recommended expiry time for ^{99m}Tc-exametazime is 30 minutes after preparation, statistical analysis by unpaired Student's t-test was confined to the 2 and 30 minute results. At 2 minutes after preparation, the radiochemical purities of ^{99m}Tc-exametazime prepared according to techniques 2 and 3 did not differ significantly ($P > 0.50$) from that prepared according to technique 1. However, at 30 minutes, the radiochemical purity of ^{99m}Tc-exametazime prepared with 6 hour-old sodium pertechnetate [^{99m}Tc] was significantly lower ($P < 0.01$) whereas the radiochemical purity of ^{99m}Tc-exametazime prepared with 6 hour-old sodium pertechnetate [^{99m}Tc] + sodium iodide was not significantly different.

In summary, HPLC has been used to demonstrate that when sodium pertechnetate [^{99m}Tc]

for use in the preparation of ^{99m}Tc-exametazime is supplied in a sodium iodide kit, its shelf-life can be extended from the recommended 2 hours to at least 6 hours.

6.4 Effect of sodium pertechnetate [^{99m}Tc] source on the stability of ^{99m}Tc-exametazime.

As discussed earlier in this chapter, the 2 hour restriction on the age of sodium pertechnetate [^{99m}Tc] used in the preparation of ^{99m}Tc-exametazime can present a considerable, if not insurmountable, problem. In an examination of this problem, Ponto (1990) has challenged the 2 hour restriction by publishing data which show that when five hour-old sodium pertechnetate [^{99m}Tc] from ^{99m}Tc generators manufactured by Mediphysics or Mallinckrodt is used, ^{99m}Tc-exametazime of satisfactory radiochemical purity is obtained. Although it is suggested, it is not clear from the publication of Ponto (1990) if these apparently favourable findings are a result of some characteristic of the sodium pertechnetate [^{99m}Tc] that is obtained from Mediphysics and Mallinckrodt ^{99m}Tc generators. Also, the conclusion is based solely on measurements made immediately after reconstitution. Of much greater importance is the radiochemical purity at the time that the ^{99m}Tc-exametazime is administered to the patient. Therefore, unless analysis and administration are performed simultaneously, the radiochemical purity of the ^{99m}Tc-exametazime that is injected into the patient is unknown. In practice, there are many circumstances which delay the administration of a medicine to a patient and make it impossible to guarantee that ^{99m}Tc-exametazime will always be administered immediately after reconstitution of the kit. It is therefore of much greater value to know that the method of preparation results in a satisfactory radiochemical purity at the expiry time quoted for the radiopharmaceutical. In the case of ^{99m}Tc-exametazime, this is 30 minutes after preparation.

In the work on the use of sodium iodide kits described earlier, I confirmed that the effect reported by Ponto (1990) is also seen when sodium pertechnetate [^{99m}Tc] from a generator manufactured by Amersham is used to prepare ^{99m}Tc-exametazime, i.e. radiochemical purity is high immediately after preparation regardless of the age of the sodium pertechnetate [^{99m}Tc]. However, when 6 hour-old sodium pertechnetate [^{99m}Tc] is used, the radiochemical purity of the ^{99m}Tc-exametazime at 30 minutes after preparation is significantly lower than when 1 hour-old sodium pertechnetate [^{99m}Tc] is used.

I therefore used HPLC in the following study to establish a) if the phenomenon reported by Ponto (1990) is peculiar to sodium pertechnetate [^{99m}Tc] that is obtained from the generators used in his study or if it is observed when ^{99m}Tc from other generators is used, and b) if the reported insignificance of eluate age is valid at the 30 minute expiry time of ^{99m}Tc-exametazime.

6.4.1 Preparation of ^{99m}Tc-exametazime

The Mediphysics and Mallinckrodt ^{99m}Tc generators that were used in the study of Ponto (1990) are not available in Europe. Mallinckrodt does, however, manufacture a ^{99m}Tc generator in Europe. The ^{99m}Tc generators used in this study were therefore the 8.5 GBq UltraTechneKow (Product Code DRN 4329, Mallinckrodt) and the 15 GBq Amertec II (Product Code MCC20, Amersham). The generators were eluted 24 hours before being eluted to provide the sodium pertechnetate [^{99m}Tc] that was used in the preparation of ^{99m}Tc-exametazime. Eluates from each manufacturer's generators were diluted immediately after elution as in the previous experiment. ^{99m}Tc-exametazime injections (1.1 GBq/5 ml) were prepared according to the following two techniques:

1. ***1 hour-old sodium pertechnetate [^{99m}Tc]:*** The volume of generator eluate that contained 1.3 GBq of ^{99m}Tc was diluted to 5.5 ml with Sodium Chloride Injection B.P. and injected into a sterile 10 ml nitrogen-filled vial (Product Code N46, Amersham). One hour later, 5.0 ml of this solution was injected into a Ceretec kit.

2. ***6 hour-old sodium pertechnetate [^{99m}Tc]:*** The volume of generator eluate that contained 2.4 GBq of ^{99m}Tc was diluted to 5.5 ml with Sodium Chloride Injection B.P. and injected into a sterile 10 ml nitrogen-filled vial (Product Code N46, Amersham). Six hours later, 5.0 ml of this solution was injected into a Ceretec kit.

Six ^{99m}Tc-exametazime injections were prepared by each of the two techniques. A different lot of Ceretec kits was used for each set of four experiments, i.e. reconstitution with 1 hour-old and 6 hour-old sodium pertechnetate [^{99m}Tc] from each manufacturer's generator. For the experiments involving sodium pertechnetate [^{99m}Tc] from the Amersham generator, four results from the previous experiment were used since vials from the same batches of Ceretec

kits were available to carry out paired experiments with sodium pertechnetate [^{99m}Tc] from the Mallinckrodt generator. Two fresh experiments were necessary since vials from the batches of Ceretec kits used in the earlier study were no longer available. Reconstituted kits were stored at room temperature.

6.4.2 Measurement of radiochemical purity

At 2, 30 and 60 minutes after reconstitution of each kit, the radiochemical purity of the ^{99m}Tc-exametazime was measured by HPLC.

As in previous experiments, the mean recovery from the column was satisfactorily high ($99.1 \pm 2.7\%$, $n=72$). The results of the radiochemical analyses are shown in Table 6.4. The levels of unidentified species and secondary ^{99m}Tc-exametazime complex were similar to those measured in the previous experiment and were similar in ^{99m}Tc-exametazime prepared using ^{99m}Tc from either manufacturer's generator.

Regardless of whether 1 hour-old or 6 hour-old sodium pertechnetate [^{99m}Tc] from either manufacturer's generator was used to prepare ^{99m}Tc-exametazime, radiochemical purity was high at 2 minutes after preparation. Analysis by unpaired Student t-test showed no significant differences ($P>0.05$). This agrees with the finding of Ponto (1990). However, at 30 and 60 minutes after preparation, the radiochemical purities of the ^{99m}Tc-exametazime prepared using 6 hour-old sodium pertechnetate [^{99m}Tc] were significantly lower ($P<0.05$) than those prepared with 1 hour-old sodium pertechnetate [^{99m}Tc]. Similar results were found for each manufacturer's generator. No significant differences in radiochemical purity were found between corresponding results from ^{99m}Tc-exametazime prepared with sodium pertechnetate [^{99m}Tc] from each manufacturer's generator.

Of the impurities in ^{99m}Tc-exametazime, ^{99m}Tc-pertechnetate was the only one that was found to differ as a result of the age of the sodium pertechnetate [^{99m}Tc] used. The changes in the levels of ^{99m}Tc-pertechnetate impurity with time are shown diagrammatically in Figure 6.6. When 1 hour-old sodium pertechnetate [^{99m}Tc] was used, the levels of ^{99m}Tc-pertechnetate impurity rose slowly over the one hour of the experiment. In contrast, when 6 hour-old sodium pertechnetate [^{99m}Tc] was used, the levels rose much more rapidly. Comparable results were obtained when sodium pertechnetate [^{99m}Tc] from either manufacturer's generator was used.

Table 6.4 Effect of age of sodium pertechnetate [^{99m}Tc] from two generators on the composition of ^{99m}Tc-exametazime.

Sodium pertechnetate [^{99m}Tc]	*Species (peak no.)*	*% of total activity at times after preparation*					
		Amersham generator			*Mallinckrodt generator*		
		2 min	*30 min*	*60 min*	*2 min*	*30 min*	*60 min*
1 hour-old	Unknown 1 (I)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
	^{99m}Tc-pertech (II)	1.2 ± 0.2	1.8 ± 0.8	2.9 ± 2.1	1.6 ± 0.8	1.3 ± 0.3	2.1 ± 0.5
	2° ^{99m}Tc-examet (III)	2.8 ± 0.3	6.1 ± 1.3	9.5 ± 3.0	3.0 ± 0.7	6.9 ± 0.9	9.9 ± 0.9
	Unknown 2 (IV)	3.2 ± 1.0	3.5 ± 1.2	4.2 ± 1.4	2.3 ± 0.4	3.0 ± 0.4	3.2 ± 0.5
	1° ^{99m}Tc-examet (V)	92.7 ± 1.2	88.5 ± 2.1	83.2 ± 3.7	92.9 ± 1.5	88.7 ± 0.7	84.6 ± 0.6
	Recovery	100.1 ± 1.0	99.2 ± 2.4	98.7 ± 1.9	98.6 ± 1.8	98.2 ± 3.6	98.0 ± 2.7
6 hour-old	Unknown 1 (I)	0.1 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
	^{99m}Tc-pertech (II)	1.8 ± 1.2	10.0 ± 2.2	17.8 ± 2.8	1.7 ± 0.4	8.4 ± 1.7	15.6 ± 2.6
	2° ^{99m}Tc-examet (III)	2.6 ± 0.9	5.6 ± 0.9	7.6 ± 1.1	3.4 ± 1.1	6.1 ± 1.1	8.5 ± 1.7
	Unknown 2 (IV)	3.0 ± 1.2	3.1 ± 1.0	3.2 ± 1.0	2.9 ± 0.4	3.3 ± 0.5	3.4 ± 0.6
	1° ^{99m}Tc-examet (V)	92.5 ± 2.0	80.9 ± 1.8	70.8 ± 2.9	91.8 ± 1.9	81.9 ± 2.1	72.0 ± 2.7
	Recovery	99.8 ± 1.4	100.0 ± 2.3	99.0 ± 2.7	99.6 ± 5.7	97.9 ± 2.9	100.5 ± 3.0

Each value is the mean ± standard deviation of six results

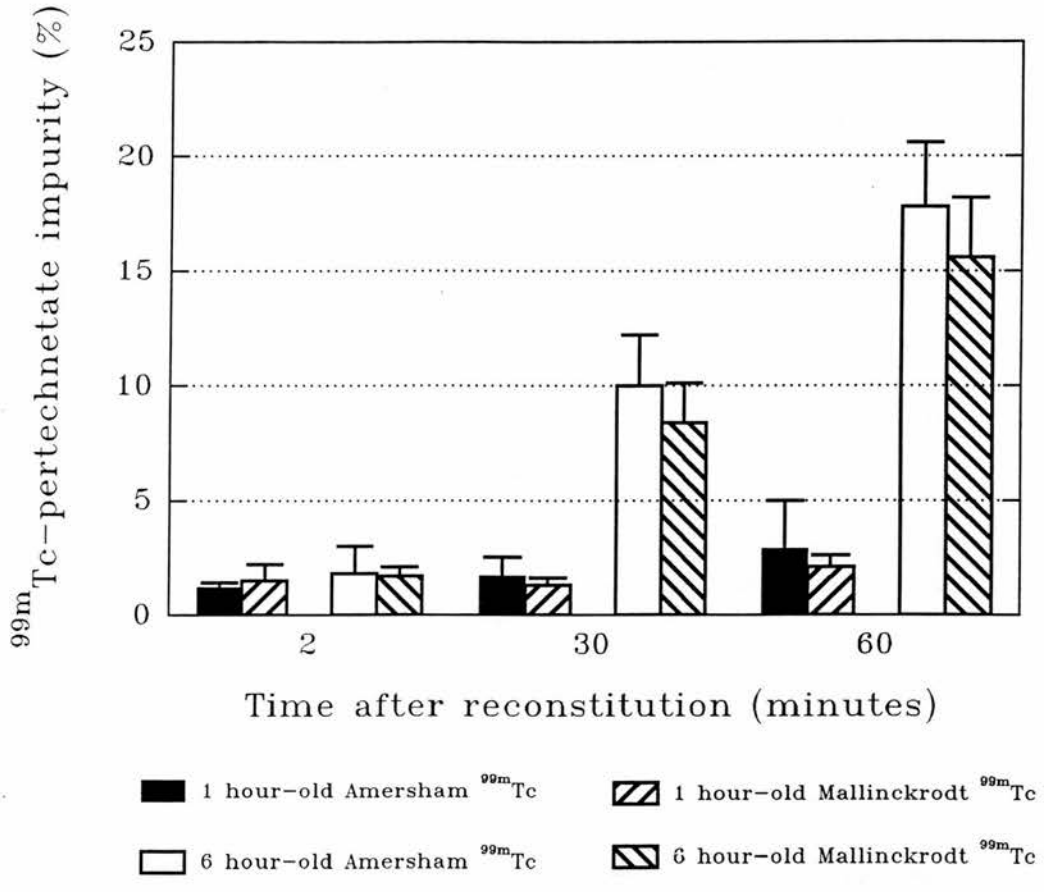

Figure 6.6 *Change in the level of ^{99m}Tc-pertechnetate impurity in ^{99m}Tc-exametazime.*

6.5 Summary

In the work described in this chapter I have:

1. validated an HPLC technique for determining the radiochemical purity of ^{99m}Tc-exametazime

2. developed a technique for preparing stable kits of sodium iodide

3. demonstrated that the sodium iodide kits can be used successfully to stabilize sodium pertechnetate [^{99m}Tc] that is dispensed for use in the preparation of ^{99m}Tc-exametazime

4. confirmed that the radiochemical stability of ^{99m}Tc-exametazime is influenced by the age of the sodium pertechnetate [^{99m}Tc] used in its preparation

5. demonstrated that the radiochemical stability of ^{99m}Tc-exametazime is not influenced by the brand of generator from which the sodium pertechnetate [^{99m}Tc] used in its preparation is obtained

From these findings, it can be concluded that HPLC is a satisfactory technique for measuring the radiochemical purity of ^{99m}Tc-exametazime.

7. Discussion

Throughout this thesis, I have discussed the specific aspects of my work in the relevant chapters. This final discussion will therefore deal with the overall implications of the studies that I have undertaken.

7.1 Design of the radiation detector

Radiation detectors for HPLC are available commercially from several sources. Although these can be used for the detection of ^{99m}Tc, they are designed primarily for use with beta-emitting radionuclides such as carbon-14 and tritium (^{3}H). Two modes of detection are used in these instruments. One involves mixing the column eluate with liquid scintillator and then passing the mixture through a flow-cell in a modified liquid scintillation counter. This arrangement requires a means by which eluate and scintillator can be mixed on-line and yet not dilute the eluate to an extent that degrades resolution to an unacceptable level. The other, more elegant arrangement is the use of a detector flow-cell which consists of a piece of plastic scintillator coupled to one or two photomultipliers - the use of two allows coincidence counting to be used. The eluate from the column is passed through a hole drilled in the scintillator. The radiation emitted from the eluate is then detected when it interacts with the plastic scintillator. The advantage of this second approach is that it avoids the complex arrangement that is required to mix liquid scintillator with the column eluate.

The complexity of these two methods of detection is a consequence of the difficulty of detecting beta-radiation. This also makes the equipment expensive. The detection of gamma-radiation such as that emitted by ^{99m}Tc is a much more straightforward task since a simple sodium iodide crystal detector can be used. This type of detector is inappropriate for the detection of beta-emitters since the beta particle has insufficient energy to pass through the metal can in which a sodium iodide crystal is typically housed. I therefore constructed a simple detector by positioning a length of column outlet tubing inside the well or across the face of a sodium iodide crystal. To accommodate the range of radioactive concentrations at which ^{99m}Tc radiopharmaceuticals are prepared, the detection efficiency could be optimised by altering the position of the tubing relative to the crystal. The resulting flow-cell volumes of between 2 and 10 μl are comparable to those found in other

HPLC detectors.

Two methods of recording the chromatograms were used. The older system based on a counter-ratemeter and printer was inexpensive but had the disadvantage of producing long print-outs if short counting times were used to capitalize on the high resolution of HPLC. Also, manual calculation of the counts in peaks was necessary. The newer system in which a computer-based multichannel analyser was used to record the chromatograms had the advantages of the data being stored on disk and automatic integration of the counts in peaks. One possible disadvantage of using a multichannel analyser was the possibility of loss of counts due to dead-time at high count-rates. With HPLC, peaks on chromatograms should be extremely sharp and this could result in high count-rates for short periods of time. However, the loss due to dead-time at the highest count-rate of 6,000 counts per second obtained in the course of my work was found to be only 0.5%. The use of the multichannel analyser was therefore considered a satisfactory and convenient means of recording chromatograms from HPLC.

7.2 Choice of ^{99m}Tc radiopharmaceuticals to be studied

Approximately thirty ^{99m}Tc radiopharmaceuticals are currently available. When embarking on a study such as the one described in this thesis, it would be unrealistic to set out with the aim of investigating the role of HPLC in relation to each one. I therefore chose three for which an aspect of radiochemical purity was of particular relevance to routine radiopharmacy practice.

^{99m}Tc-MAG_3 was a promising new radiopharmaceutical for which an impractical expiry time of one hour after preparation had been recommended by its manufacturer. The logistics and economics of using a product with such a short shelf-life were likely to preclude it from routine use. To establish conditions that result in high radiochemical purity and stability would increase the likelihood of ^{99m}Tc-MAG_3 finding a place in routine nuclear medicine practice.

^{99m}Tc-DMSA has been available for approximately 15 years but doubt over the consequences of preparing it from ^{99m}Tc with a high ^{99}Tc content remained unresolved. Also, if ^{99m}Tc-DMSA is to be used in absolute measurement of renal function, high radiochemical purity is of greater importance than if it is being used solely to obtain static

images of the kidneys or to provide a measure of relative renal function.

^{99m}Tc-exametazime is a new radiopharmaceutical of low stability. To achieve the highest possible radiochemical purity, its manufacturer specifies several criteria that must be observed during preparation. One of these is to use ^{99m}Tc-pertechnetate that has been eluted from a generator not more than two hours previously. If ^{99m}Tc-exametazime is required in a nuclear medicine department that is some distance from the radiopharmacy, a means of overcoming this requirement is necessary. A recent publication cast doubt on the necessity for this two hour restriction and this claim required further investigation.

7.3 Recovery from the HPLC column

Throughout, I have emphasized that a valid measurement of radiochemical purity is only possible if there is complete recovery of the sample from the HPLC column. When incomplete recovery occurs due to an impurity in the radiopharmaceutical being irreversibly adsorbed in the column, the impurity will remain undetected and a value for radiochemical purity that is calculated using the data recorded from an on-line radiation detector will therefore be erroneously high. Conversely, when incomplete recovery is a consequence of a proportion of the *"chemical form declared"* being adsorbed, calculation of the radiochemical purity will give an erroneously low value.

In the assay of non-radioactive compounds by HPLC, the problem of adsorption is overcome by comparing the chromatogram obtained from a sample of the compound being tested with one obtained from a pure reference sample of the compound. With this technique, adsorption on the column of a proportion of the compound should not affect the result since test and reference samples will undergo adsorption to the same extent. The adsorption on the column of an impurity in the test compound is of no consequence since the purity of the test substance is calculated from a comparison of the sizes of the peaks obtained with the test and reference samples. For example, if solutions of a reference compound and a test compound that contains an impurity which is retained on the column are prepared at equal concentrations, the compound in the reference sample will produce a larger peak than that in the test sample. The low purity of the test compound will therefore be revealed although both chromatograms will contain only one peak. When dealing with radiopharmaceuticals, there are no reference compounds available due to the inherent instability of these substances - a consequence of their radioactive nature and, in the case of

most ^{99m}Tc radiopharmaceuticals, that they are formed by reduction and are therefore susceptible to oxidation. An assay procedure similar to that used for non-radioactive compounds cannot therefore be used.

Also in the analysis of non-radioactive compounds by HPLC, an internal standard is commonly added to both unknown and reference samples. The internal standard is chosen to give a peak on the chromatogram at a retention time that does not coincide with that of the test substance or one of the impurities that might be present in the test mixture. The size of the internal standard peak is then used to normalize any fluctuations in the size of the peaks due to variations in volume of sample injected, changes in flow-rate, fluctuations in detector sensitivity, etc. This strategy cannot easily be used in the analysis of radiopharmaceuticals since, similar to reference compounds, pure radiolabelled compounds that would be suitable for use as internal standards are not available. More significantly, an internal standard is only of value if a reference substance is available.

In view of these limitations, successful analysis of ^{99m}Tc radiopharmaceuticals by HPLC is dependent upon complete recovery from the column. Several techniques can be used to measure recovery. Mathis et al. (1986) have described a technique that involves placing a length of the tubing that connects the injector to the column in the on-line radiation detector. When the injection is made, the sample passes through the detector and counts are recorded. The sample then passes along the column where it is separated into its components which pass through the detector as they are eluted. The recovery is calculated by expressing the counts detected from the eluted peaks as a percentage of the counts recorded from the injection. Unless the lengths of tubing that constitute the inlet and outlet detectors have identical volumes, a correction factor must be applied to account for the difference. This technique has the attraction of the data for the recovery calculation being acquired on-line but suffers from two main disadvantages. Firstly, the detector must be constructed in a manner which ensures that the relative positions of the two pieces of tubing in relation to the radiation detector are maintained. If this is not achieved, day-to-day differences in the efficiencies of detection will be introduced due to movement of the tubing, invalidating any correction factor that has been established. Alternatively, the relationship between the two detectors would have to be re-established periodically. If the tubing were particularly prone to movement, calibration would be necessary on each occasion that the equipment is used. Secondly, a fairly long piece of tubing between the injector and column is required if it is to be located in the same detector as the outlet tubing. Conventionally, this tubing is kept as short as possible to minimize spreading of

the sample as it travels to the column and the consequent reduction in resolution.

An extension of the above concept would be to have two detectors - one positioned over the column inlet tubing and the other over the outlet tubing. This arrangement would not require extension of the inlet tubing but each detector would require its own nucleonics and recording system. Cross-calibration of the detectors would be necessary to account for differences in detection efficiencies and the volumes of mobile phase being monitored. Equipment of this type would offer the advantage of on-line data acquisition for the recovery measurement. A disadvantage would be the cost since two detector systems and associated nucleonics would be necessary. Also, the potential for fluctuations in the sensitivities of the two systems, either due to movement of the pieces of tubing in relation to their detectors or due to instability in the sets of nucleonics, would demand a rigorous programme of cross-calibration.

A third technique that can be employed for the measurement of recovery is calibration of the on-line radiation detector for the radionuclide that is present in the radiopharmaceutical. This can be achieved with a standard solution of the radionuclide in a chemical form that has been shown to be completely recovered from the chromatographic system. For ^{99m}Tc radiopharmaceuticals, this chemical form is likely to be the pertechnetate ion since this chemical state of ^{99m}Tc is unreactive and is therefore unlikely to form a complex with the column packing material. In this technique, the standard and the radiopharmaceutical to be analysed must be prepared at known radioactive concentrations. In practice, when radiopharmaceuticals are typically prepared in rubber-capped vials and solutions are manipulated by means of disposable hypodermic syringes, accurate measurement of volumes cannot be guaranteed. An accurate measurement of radioactive concentration can therefore be achieved more easily by weighing the vial before and after addition of the liquid and expressing the radioactive concentration of the solution in MBq per gram. A sample of the standard is then injected onto the column and the counts in the detected peak are recorded. From the ratio of the radioactive concentrations of the radiopharmaceutical and standard solution, the counts that should be in the chromatogram obtained from the radiopharmaceutical are calculated. A sample of the radiopharmaceutical is then injected, the chromatogram is acquired and the counts in the chromatogram are recorded. After correction of the counts in the chromatogram for the radioactive decay that has occurred since recording of the standard chromatogram, the recovery is calculated by expressing the counts in the radiopharmaceutical chromatogram as a percentage of the counts that should be in the chromatogram. As with the two described previously, this technique has the

advantage that the data used to calculate recovery are acquired on-line in the course of the analysis. However, the technique suffers from two principal disadvantages. The first is that its validity depends on the standard being completely recovered from the column. It is possible that species adsorbed on a column from previous samples of radiopharmaceuticals might cause retention of the standard and invalidate the recovery measurement technique. This is not unlikely with ^{99m}Tc radiopharmaceuticals since a stannous containing species adsorbed on the column from a previous analysis could reduce and thereby bring about complexation and retention of ^{99m}Tc-pertechnetate that was injected as a standard for a subsequent analysis. The second disadvantage is that the times of measurement of radioactive concentrations and injection of samples must be recorded since correction for radioactive decay is necessary.

In view of the problems that exist with these techniques for measuring recovery, I opted for what is probably the simplest technique of all. This involves collecting the eluate from the column and comparing the count-rate from the eluate with the count-rate from an accurately pipetted sample of the radiopharmaceutical being analysed. As discussed in Chapter 3, this technique involves cross-calibration of the pipette and loop of the injector but unlike the technique of passing the eluent and eluate through the same detector, the correction factor established in the cross-calibration is less likely to change from day to day. Also, unlike the techniques that depend upon on-line detection for measurement of recovery, the eluate collection technique is not influenced by any changes in flow-rate that occur during the analysis.

The consistently high recoveries in the analyses of ^{99m}Tc-MAG_3 and ^{99m}Tc-exametazime indicate that this aspect of the use of HPLC to measure the radiochemical purity of these radiopharmaceuticals is satisfactory.

The low recoveries encountered throughout the work on ^{99m}Tc-DMSA demonstrate that the chromatographic conditions evaluated for the analysis of this radiopharmaceutical are unsatisfactory. During the studies on ^{99m}Tc-DMSA, the quite unexpected phenomenon of sample adsorption in the loop of the valve injector was identified as a potential cause of error with this technique for measuring recovery. However, adsorption in the loop would introduce an error in all of the recovery techniques described. In the case of the ^{99m}Tc-DMSA work, the washing of the adsorbed material onto the column by the mobile phase resulted in an apparent recovery of greater than 100%. The impossibility of this situation prompted the further investigation of the phenomenon. Had the adsorbed fraction not been

washed onto the column, a recovery of less than 100% would have resulted. Without further investigation this might have been attributed to an impurity in the radiopharmaceutical being adsorbed on the column whereas it could be caused by adsorption of a proportion of the *"chemical form declared"* in a satisfactory radiopharmaceutical, thereby giving a false indication of low radiochemical purity. Column scanning would have shown the absence of adsorbed ^{99m}Tc on the column and would thereby have prompted an investigation of the reason for low recovery.

7.4 Characterization of the species responsible for the principal peak

A potential limitation of using HPLC for the analysis of ^{99m}Tc radiopharmaceuticals is the difficulty, if not impossibility, in demonstrating that the main peak of the chromatogram contains only the *"chemical form declared"*. This problem is a consequence of the extremely low mass of the radiolabelled species that is present in radiopharmaceuticals. Although radiopharmaceutical kits typically contain milligram quantities of the compounds to be labelled, only a small fraction becomes associated with ^{99m}Tc. For example, the Amersham Ceretec kit contains 500 μg of exametazime. From the structure of the ^{99m}Tc-exametazime complex it can be calculated the if the kit is reconstituted with the maximum 1.11 GBq of ^{99m}Tc, only 20 ng of ^{99m}Tc-exametazime is formed. This ^{99m}Tc-exametazime is contained in the reconstitution volume of 5 ml. A 20 μl sample that might typically be injected onto an HPLC column therefore contains only 80 pg of radiolabelled compound. With such a low mass of material being responsible for the peak on the chromatogram, identification of the structure of the compound is difficult, if not impossible. Radiolabelled impurities are likely to be present in considerably lower concentration than the radiopharmaceutical species. Identification of their structures presents even greater difficulties.

In the analysis of non-radioactive compounds, this problem is much less of a limitation. Firstly, compounds tend to be present in much higher concentrations and techniques such as the various forms of spectroscopy, x-ray crystallography and elemental analysis can be used to determine structure. Secondly, if two species are responsible for a single peak on a chromatogram obtained with an ultraviolet (UV) detector, it is unlikely that they will have identical absorptive properties. Comparison of the peak with that from a standard of the compound under test will show a discrepancy. The presence of two or more species that co-elute from the column can also be demonstrated with a diode-array UV detector

providing the species have sufficiently different absorption spectra. None of this is possible with a radiation detector since the property being detected, i.e. the radiation that is emitted by the radionuclide, is common to all the radiolabelled species present in the radiopharmaceutical. Also, as discussed previously no standards are available against which the radiopharmaceutical can be compared.

HPLC is not alone in suffering from the difficulty of demonstrating that the measurement that is taken to represent the *"chemical form declared"*, is due solely to that species. All chromatographic techniques have this limitation. As discussed in Chapter 1, thin-layer and paper chromatographic techniques in which the radiolabelled species either remain at the origin or travel with the solvent front and gel filtration techniques in which the radiolabelled species elute at either the void volume or total volume of the column are particularly prone to producing misleading results due to an impurity exhibiting the same behaviour as the species for which the analysis is being undertaken.

For the analysis of ^{99m}Tc radiopharmaceuticals it is therefore necessary to rely upon the high resolution of which HPLC is capable and assume that impurities do not co-elute with the principal species. It is important, however, to ensure that the principal species does not elute with the retention time of an unretained solute. This situation is analogous to a species that migrates with the solvent front in thin-layer and paper chromatography or elutes at the void volume in gel filtration. More than one constituent of a radiopharmaceutical can exhibit this behaviour. In an HPLC technique that is based on the principal species behaving in this way, there is every likelihood that one or more impurities will co-elute with the principal species. The technique will therefore lead to an over-estimate of radiochemical purity due to its lack of specificity. Early reports of the use of HPLC for the analysis of ^{99m}Tc radiopharmaceuticals are open to criticism on these grounds (Russell & Majerik, 1979, Wong et al., 1981). For example, Wong et al. (1981) reported that with their chromatographic system of an ODS column and phosphate buffer/acetonitrile mobile phase, ^{99m}Tc-methylene diphosphonate and ^{99m}Tc-pertechnetate impurity eluted with retention volumes of 2 ml and 3 ml respectively. From the dimensions quoted for the column and the fact that two other radiopharmaceuticals (^{99m}Tc-DTPA and ^{99m}Tc-pyrophosphate) eluted with retention volumes of 2 ml, it is a reasonable assumption that this represents the volume for an unretained solute. Many authors, e.g. Srivastava et al. (1982), Hoch & Pinkerton (1986) and Huigen et al. (1988), have shown that ^{99m}Tc-methylene diphosphonate contains several radiolabelled species. The conditions used by Wong et al. (1981) are incapable of resolving these species as they co-elute as unretained

solutes. From these results, Wong et al. (1981) concluded that their HPLC conditions offered "marginal utility" for these radiopharmaceuticals.

In none of the techniques that I used to measure radiochemical purity did the principal species elute as an unretained species. The principal peaks obtained with ^{99m}Tc-MAG$_3$ and ^{99m}Tc-exametazime were well separated from the other peaks in the chromatogram and the assumption was made that these represented the *"chemical form declared"*. In the case of ^{99m}Tc-DMSA, the principal species was retained on the column and the technique was of no value for determination of radiochemical purity.

7.5 Comparison of HPLC with other chromatographic techniques

One of the ways in which I have validated HPLC as a satisfactory technique for measuring the radiochemical purity of ^{99m}Tc radiopharmaceuticals is to compare the results with those obtained using other chromatographic techniques. I have shown that measurement of the radiochemical purity of ^{99m}Tc-exametazime gives results that are in good agreement with those obtained using a combination of thin-layer and paper chromatography. From these findings I have concluded that HPLC is a satisfactory technique for measuring the radiochemical purity of this radiopharmaceutical. A parallel conclusion that could be drawn from my results and which would be equally valid is that the thin-layer and paper chromatography techniques are satisfactory for determining radiochemical purity. This would be a valuable conclusion since these techniques are more easily performed, much cheaper and therefore more widely used than HPLC. This situation demonstrates a drawback of validating a chromatographic technique by comparing it to another chromatographic technique - there is no certainty that either gives the correct result. It is possible for an impurity to be present in a radiopharmaceutical and for that impurity to behave in an identical manner to the radiopharmaceutical species in all chromatographic systems. With this possibility in mind, the advantage of HPLC is its much higher specificity and resolution than the other chromatographic techniques that are commonly used for radiopharmaceuticals. HPLC therefore gives the best opportunity for the detection of impurities that might be present in radiopharmaceuticals.

My work on ^{99m}Tc-MAG$_3$ illustrates the above concept. The species (peak 6 on Figure 4.9) which elutes after the main peak behaves on the thin-layer and paper chromatography systems in a manner similar to ^{99m}Tc-MAG$_3$. While all three techniques give similar,

although statistically different, values of radiochemical purity when the level of this impurity is small, they would not if the level were greater. In this situation, thin-layer and paper chromatography would give erroneously high values of radiochemical purity. For this radiopharmaceutical, therefore, comparison of HPLC with other chromatography techniques has served to demonstrate its superiority due to its ability to separate the impurities from the *"chemical form declared"*.

The samples of ^{99m}Tc-MAG_3 and ^{99m}Tc-exametazime that I used in my investigations were predictable in terms of the impurities that were present. However, as I discussed in Chapter 1, there have been many reports of problems with ^{99m}Tc radiopharmaceuticals that have been caused by seemingly innocuous factors. In the measurement of radiochemical purity it is therefore important to detect all the impurities that are present. It should not be a case of using a technique that detects only the predictable impurities but using a technique that will detect any impurity that is present. HPLC should provide the best opportunity of achieving this aim.

7.6 Summary

With reference to the objectives that I set in Chapter 1, the work described in this thesis can be summarized as follows:

An on-line HPLC detector for use with ^{99m}Tc radiopharmaceuticals has been constructed and shown to function satisfactorily.

An HPLC technique has been developed and shown to be satisfactory for measurement of the radiochemical purity of ^{99m}Tc-MAG_3. The technique has been used to demonstrate the stability of this radiopharmaceutical, previously reported as being unstable, and to investigate effects of different preparative conditions on radiochemical purity and stability.

No satisfactory technique for measurement of the radiochemical purity of ^{99m}Tc-DMSA has been established. However, several important facts relevant to the routine preparation of this radiopharmaceutical have been discovered: no evidence has been found to suggest that the levels of ^{99}Tc encountered in routine radiopharmacy practice have a deleterious effect on

the radiochemical purity of ^{99m}Tc-DMSA; for the first time, ^{99m}Tc(V)-DMSA has been shown to be a common impurity in ^{99m}Tc(III)-DMSA; the "wet labelling" technique is unsatisfactory for the preparation of ^{99m}Tc-DMSA; the concentration of ^{99m}Tc(V)-DMSA in ^{99m}Tc-DMSA is influenced by the volume in which the DMSA kit is reconstituted.

An HPLC technique has been developed and shown to be satisfactory for measurement of the radiochemical purity of ^{99m}Tc-exametazime. The technique has been used to develop a routine method for using sodium iodide kits to stabilize ^{99m}Tc-pertechnetate that is dispensed for the preparation of ^{99m}Tc-exametazime and to confirm that the radiochemical stability of ^{99m}Tc-exametazime is influenced by the age of the ^{99m}Tc-pertechnetate used in its preparation.

The non-silica HPLC columns PLRP-S and PRP-1 have been found to be of value in the analysis of ^{99m}Tc-DMSA and ^{99m}Tc-exametazime respectively. Hypercarb has not been found to be of value although it was only investigated for the analysis of ^{99m}Tc-DMSA.

In accordance with the aim set at the start of this work, quantitative HPLC has been shown to be a satisfactory technique for determining the radiochemical purity of ^{99m}Tc radiopharmaceuticals.

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Appendix I - Names and addresses of suppliers

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Amersham International plc	White Lion Road Amersham HP7 9LL
Anachem Ltd	20 Charles Street Luton Bedfordshire LU2 0EB
Canberra Nuclear Data	Wessex Road Bourne End Buckinghamshire SL8 5DU
Datac Ltd	Tudor Road Broadheath Altrincham WA14 5TN
Denley	Daux Road Billinghurst Sussex RH14 9SJ

Gelman Sciences Ltd	10 Harrowden Road Brackmills Northampton NN4 0EZ
Hamilton Company	P.O. Box 10030 Reno Nevada 89520-0012 U.S.A.
Hewlett-Packard Ltd	Coin Road Bracknell RG12 1HN
IBM United Kingdom	PO Box 41 North Harbour Portsmouth PO6 3AU
Jandel Scientific	65 Koch Road Corte Madera CA 94925 U.S.A.
J & P Engineering Ltd (this company is no longer trading)	Cardiff Road Reading RG1 8JF
Linseis GMBH	Vielitzer Strasse 43 8672 Selb Germany
LKB Wallac	P.O. Box 10 20101 Turku 10 Finland

Mallinckrodt Medical (UK) Ltd	11 North Portway Close Round Spinney Northampton NN3 4RQ
E. Merck	D-6100 Darmstadt Germany
Nuclear Enterprises	Bath Road Beenham Reading
Perkin-Elmer Ltd	Post Office Lane Beaconsfield Buckinghamshire HP9 1QA
Philips Scientific	York Street Cambridge CB1 2PX
Polymer Laboratories Ltd	Church Stretton Shropshire SY6 6AX
Rheodyne Inc	P.O. Box 996 Cotati California 94928 U.S.A.
Scintronix Ltd (this company is no longer trading)	1 Drummond Square Brucefield Industrial Estate Livingston EH54 9DH

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Unisoft Ltd	P.O. Box 383 London N6 5UP
Whatman Scientific Ltd	St Leonard's Road 20/20 Maidstone Kent ME16 0LS

Appendix II - Published papers and abstracts

Millar A M, Wilkinson A G, McAteer E, Best J J K (1988) $^{99}Tc^{m}$-MAG3: in-vitro stability and in-vivo behaviour at different times after preparation (abstract). *Nuclear Medicine Communications* 9: 190.
Oral presentation at the Annual Meeting of the British Nuclear Medicine Society, April 1988, London.

Millar A M, Wilkinson A G, McAteer E, Best J J K (1990) $^{99}Tc^{m}$-MAG3: *in vitro* stability and *in vivo* behaviour at different times after preparation. *Nuclear Medicine Communications* 11: 405-412.

Millar A M and O'Brien L M (1990) An investigation of factors that might influence the radiochemical purity and stability of ^{99m}Tc-MAG3. *European Journal of Nuclear Medicine* 16: 615-619.

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Millar A M (1992) A routine method for using sodium iodide to stabilize sodium pertechnetate [$^{99}Tc^{m}$] dispensed for the preparation of $^{99}Tc^{m}$-exametazime. *Nuclear Medicine Communications* 13: 306-311.

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Nuclear Medicine Communications **11**, 405–412 (1990)

$^{99}Tc^{m}$-MAG3: *in vitro* stability and *in vivo* behaviour at different times after preparation

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Summary

A kit for preparing technetium-99m mercaptoacetyltriglycine ($^{99}Tc^{m}$-MAG3), a new radiopharmaceutical for gamma camera renography, is available commercially. A drawback to the use of this kit is the recommended 1h expiry for $^{99}Tc^{m}$-MAG3. This short expiry is a consequence of the possible growth of an impurity which undergoes hepatobiliary excretion and might interfere with renal imaging.

Radiochemical purity of $^{99}Tc^{m}$-MAG3 was measured by high performance liquid chromatography at 0, 1 and 6 h after preparation and was found to be consistently > 95. $^{99}Tc^{m}$-MAG3 was shown to contain five impurities, one of which increased from 0.5% to 1% over 6 h. Dilution of $^{99}Tc^{m}$-MAG3 eliminated this effect.

A two-part clinical study was undertaken. For Part I, $^{99}Tc^{m}$-MAG3 was prepared at 400 MBq/4 ml. For Part II, $^{99}Tc^{m}$-MAG3 was prepared at 1 GBq/4 ml then subdivided and diluted to give single doses of 175 MBq/2.5 ml. In both parts, 10 patients were injected within 1 h after preparation and 10 were injected 5–6 h after preparation. From gamma camera images of the abdomen acquired 30 min after injection, the % injected $^{99}Tc^{m}$ in gall bladder and liver were calculated. In both parts, the % injected $^{99}Tc^{m}$ in gall-bladders and livers of the 1 h group were compared with those in the 5–6 h group and not found significantly different ($p > 0.05$).

In conclusion, $^{99}Tc^{m}$-MAG3 prepared according to the methods described, can be used up to 6 h after preparation.

Introduction

Technetium-99m mercaptoacetyltriglycine ($^{99}Tc^{m}$-MAG3) has been developed as a radiopharmaceutical for gamma camera renography [1] and a number of publications have shown it to be a suitable replacement for ^{123}I- and ^{131}I-iodohippurate and $^{99}Tc^{m}$-DTPA in the investigation of renal function [2–6]. In response to those encouraging results, a kit for the preparation of $^{99}Tc^{m}$-MAG3 has been developed (Mallinckrodt) and is now commercially available. A severe drawback to the routine use of this kit is

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the manufacturer's recommended expiry of 1 h after preparation. The reason for this short expiry time is the potential for the formation of radiochemical impurities in the $^{99}Tc^{m}$-MAG3 with time. These impurities are excreted through the hepatobiliary system and might therefore interfere with renal investigations because, in the posterior view, the gall bladder can lie within the region of interest created for the right kidney.

It was therefore decided to investigate the stability of the $^{99}Tc^{m}$-MAG3 prepared in this department to determine the level of impurities, how these levels change with time and the effect of the impurities on dynamic renal imaging in patients.

Materials and methods

Preparation of $^{99}Tc^{m}$-MAG3

Preparation of $^{99}Tc^{m}$-MAG3 for Part I of this study was performed strictly according to the manufacturer's instructions. Under aseptic conditions, sodium pertechnetate [$^{99}Tc^{m}$] injection EP (> 400 MBq/ml) from a $^{99}Tc^{m}$ generator (Amersham, Code MCC20) was diluted to 4 ml with sodium chloride injection EP and injected into a MAG3 kit (Mallinckrodt, Code DRN 4334). The vial was placed in a boiling water bath for 10 min then cooled in a water bath at room temperature for 10 min.

For Part II of the study, the $^{99}Tc^{m}$-MAG3 was prepared as above but at a radioactive concentration of 1 GBq/4 ml. Individual patient doses containing 175 MBq/2.5 ml were then dispensed by withdrawing a 0.7 ml aliquot from the kit, diluting it to 2.5 ml with sodium chloride injection EP and injecting the solution into a sterile 10 ml vial containing one nitrogen atmosphere (Amersham, Code N46).

High performance liquid chromatography

The radiochemical purity of $^{99}Tc^{m}$-MAG3 was measured by high performance liquid chromatography (HPLC) using a method provided by the manufacturer of the MAG3 kit (Mallinckrodt, personal communication). The equipment consisted of a dual piston recriprocating pump, a loop-valve injector fitted with a 20 μl loop, a 250×5 mm 5 μm Hypersil ODS column (Shandon) and a gamma radiation detector. The detector was constructed of 2 μl loop of stainless steel tubing (0.25 mm internal diameter) positioned inside a well-type sodium iodide detector coupled to a nucleonic counting system with outputs to both a chart recorder and a digital printer. The column was eluted at 1 ml min^{-1} with ethanol/0.01 M phosphate buffer pH6 (5 : 95) for 10 min then methanol/water (90 : 10) for 10 min. The counts in each peak were calculated from the output of the digital printer and expressed as a percentage of the total counts detected. The recovery of $^{99}Tc^{m}$ from the HPLC column was determined by collecting the eluate and comparing its count-rate with the count-rate from a 20 μl sample of the $^{99}Tc^{m}$-MAG3 under test. All preparations of $^{99}Tc^{m}$-MAG3 were stored at room temperature and radiochemical purity was measured at 0, 1 and 6 h after labelling. Three lots of kits prepared at 400 MBq/4 ml according to the manufacturer's instructions and one lot prepared at 1 GBq/4 ml followed by subdivision and dilution were each tested on five occasions.

Clinical study

Twenty patients attending for routine diuretic renography were studied in Part I of this trial in which $^{99}Tc^{m}$-MAG3 prepared at 400 MBq/4 ml was used. Each patient was injected with 100 MBq $^{99}Tc^{m}$-MAG3. Ten patients were injected within 1 h after preparation of the $^{99}Tc^{m}$-MAG3 while the other 10 were injected between 5 and 6 h after preparation. The routine imaging study was performed, taking 30 min. Anterior and right lateral static images of the

abdomen were then acquired in the gamma camera system's computer to access activity in the liver and gall bladder. Urine was collected from six patients and analysed by HPLC to determine the form in which the $^{99}Tc^{m}$ is excreted. A venous blood sample was taken from each patient for measurement of plasma creatinine.

In Part II of this trial, a further 20 patients were studied in an identical manner except that the $^{99}Tc^{m}$-MAG3 prepared at 1 GBq/4 ml followed by subdivision and dilution was used.

Interpretation of computer images

The percentages of injected $^{99}Tc^{m}$ in the gall bladder and liver at the end of the dynamic study were calculated from the static images using the following technique. From the anterior image, the depth of the gall bladder in the right lateral projection was measured. On the right lateral image, four regions of interest (ROI) were created around a background area, the gall bladder, the parts of the liver which did not overlap gall bladder or residual renal activity and the whole liver. The counts and number of pixels in each of the first three ROIs, and the number of pixels in the whole liver ROI were obtained. The counts in the gall bladder and liver ROIs were corrected for background. The activity in the gall bladder was then calculated by comparing the counts in the gall bladder ROI to the counts from a $^{99}Tc^{m}$ source of known activity positioned at a distance from the face of the gamma camera equal to the depth of the gall bladder. Knowing the activity of $^{99}Tc^{m}$-MAG3 injected into the patient, the % injected $^{99}Tc^{m}$ in the gall bladder at the end of the study was calculated. The % injected $^{99}Tc^{m}$ in the liver was measured in the same way except that to estimate the counts in the whole liver, it was necessary to multiply the counts in the part liver ROI by the ratio of the pixels in the whole liver ROI to the part liver ROI.

Statistical analysis

Wilcoxon's nonparametric test for unpaired comparison was used for statistical analysis. Values at $p > 0.05$ were considered to be not significant.

Results

A typical chromatogram of $^{99}Tc^{m}$-MAG3 is shown in Fig.1. The chromatogram shows six peaks with four impurity peaks (1–4) appearing before the principal $^{99}Tc^{m}$-MAG3

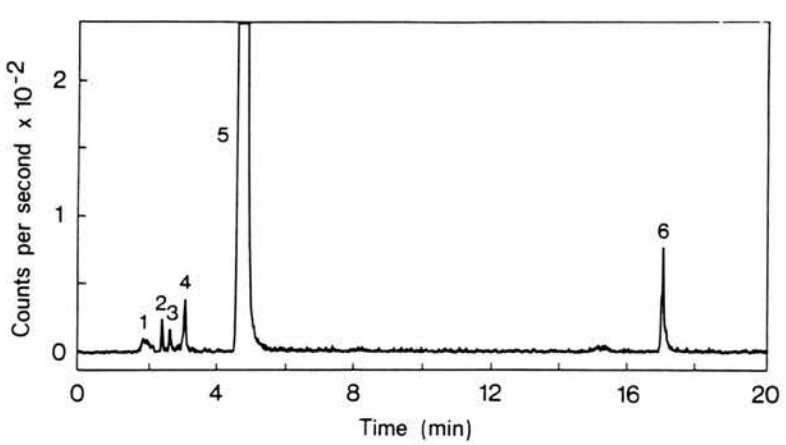

Fig. 1. High performance liquid chromatogram of $^{99}Tc^{m}$-MAG3.

Table 1. Results of the chromatographic analysis of $^{99}Tc^{m}$-MAG3 prepared at 400 MBq/4 ml.

Lot no.	Time after preparation (h)	% of the recovered activity in each peak						% recovery from column
		1	2	3	4	5	6	
8056	0	0.2±0.2	0.3±0.1	0.6±0.5	0.8±0.1	95.2±0.7	2.9±0.8	100.0±3.2
	1	0.3±0.3	0.3±0.2	0.5±0.4	1.2±0.1	94.4±1.2	3.3±0.9	96.3±3.4
	6	0.3±0.1	0.3±0.1	0.9±0.6	1.6±0.2	94.2±0.7	2.7±0.7	99.0±3.8
8068	0	0.2±0.1	0.3±0.1	0.6±0.4	0.5±0.1	95.9±0.7	2.5±0.4	100.1±0.9
	1	0.2±0.1	0.3±0.1	0.7±0.3	0.7±0.1	95.7±0.6	2.4±0.3	100.5±0.2
	6	0.2±0.1	0.3±0.1	0.9±0.4	1.1±0.1	95.2±0.7	2.3±0.2	100.4±0.1
8073	0	0.2±0.1	0.2±0.1	0.4±0.2	0.5±0.1	96.5±0.3	2.2±0.2	100.7±1.1
	1	0.2±0.1	0.2±0.0	0.5±0.2	0.7±0.1	96.4±0.2	2.0±0.1	100.8±1.7
	6	0.2±0.1	0.3±0.1	0.5±0.2	1.2±0.2	96.0±0.4	1.8±0.3	100.1±4.7

Each value is the mean ± S.D. of 5 results.

peak (5) and then a further impurity peak (6) after the change of solvent. Peak 3 was shown to have the same retention time as $^{99}Tc^{m}$-pertechnetate.

The results of the stability studies performed on the three lots of MAG3 kits prepared according to the manufacturer's instructions are shown in Table 1. Of the impurity peaks, the only one which changes significantly over the 6 h is peak 4 which approximately doubles in each case. This increase in peak 4 is accompanied by a corresponding decrease in the % activity in the main $^{99}Tc^{m}$-MAG3 peak (5). The other impurity peaks remain constant throughout the 6 h. Table 1 also shows the recoveries of $^{99}Tc^{m}$ from the HPLC column. All recoveries are in excess of 95%.

The results of the stability study performed on the $^{99}Tc^{m}$-MAG3 prepared at 1 GBq/ml followed by subdivision and dilution are shown in Table 2. The results are

Table 2. Results of the chromatographic analysis of $^{99}Tc^{m}$-MAG3 prepared at 1 GBq/4 ml followed by subdivision and dilution.

Lot no.	Time after preparation (h)	% of the recovered activity in each peak						% recovery from column
		1	2	3	4	5	6	
8073	0	0.1±0.1	0.3±0.1	0.8±0.2	0.4±0.1	96.1±0.3	2.3±0.2	97.8±1.5
	1	0.2±0.1	0.3±0.1	0.9±0.4	0.5±0.1	96.0±0.4	2.2±0.3	97.9±2.2
	6	0.1±0.1	0.3±0.1	1.1±0.2	0.4±0.1	95.7±0.3	2.4±0.1	98.0±2.6

Each value is the mean ± S.D. of 5 results.

Table 3. Results of the clinical study.

		Time between preparation and injection			
		Part I – undiluted		*Part II – diluted*	
		<1h	*5–6h*	*<1h*	*5–6h*
No. of images showing gall bladder		5	5	3	4
% injected $^{99}Tc^{m}$ in gall bladder	median (range)	0.1 (0.0–0.3)	0.1 (0.0–0.5)	0.0 (0.0–0.1)	0.0 (0.0–0.4)
% injected ^{99}Tc m in liver	median (range)	4.8 (3.1–12.9)	4.4 (2.0–8.9)	4.7 (1.4–12.3)	4.0 (2.3–12.4)
creatinine (μmol/l)	median (range)	90 (66–529)	106 (79–230)	97 (63–290)	95 (63–324)
No. of patients with creatinine <150 μmol/l		1	1	2	2

very similar to those shown in Table 1 for $^{99}Tc^{m}$-MAG3 prepared according to the manufacturer's instructions. In this case, however, the % activity in peak 4 does not double over the 6 h. The recoveries from the HPLC column are again very high.

The incidence of visualization of the gall bladder and the percentages of administered activity in the gall bladder and liver 30 min after administration of $^{99}Tc^{m}$-MAG3 are shown in Table 3. In both parts of the study, the differences in gall bladder and liver activities in the < 1 h group and the 5–6 h group were found not to be significant. Similarly, the differences between undiluted $^{99}Tc^{m}$-MAG3 administered within 1 h of preparation and diluted $^{99}Tc^{m}$-MAG3 administered 5–6 h after preparation were not significant.

Table 4 shows the HPLC profile of the $^{99}Tc^{m}$ in the urine of six patients along with the profile of a preparation of $^{99}Tc^{m}$-MAG3 for comparison. The two profiles do not differ markedly.

Table 4. Results of the chromatographic analysis of urine compared to a typical preparation of $^{99}Tc^{m}$-MAG3.

	% of the recovered activity in each peak						*% recovery from column*
Sample	*1*	*2*	*3*	*4*	*5*	*6*	
Urine (n = 6)	0.1±0.1	0.2±0.0	0.3±0.2	0.4±0.1	97.8±0.6	1.3±0.3	101.0±2.0
Lot 8068 – 1 h	0.2±0.1	0.3±0.1	0.7±0.3	0.7±0.1	95.7±0.6	2.4±0.3	100.5±0.2

Discussion

A compound labelled with $^{99}Tc^m$ and with the biological characteristics of o-iodohippurate has, for many years, been seen as the ideal radiopharmaceutical for dynamic renal imaging. With this aim in mind, a number of compounds such as $^{99}Tc^m$-DADS [7], $^{99}Tc^m$-CO_2DADS [8], $^{99}Tc^m$-thiodiglycolic acid [9] and $^{99}Tc^m$-PAHIDA [10] have been developed but none have proved to be a suitable replacement for the o-iodohippurates. The compound to be developed most recently is $^{99}Tc^m$-MAG3 which shows great promise as a replacement for iodohippurate. A number of publications have described comparisons between $^{99}Tc^m$-MAG3 and ^{123}I- or ^{131}I-iodohippurate and there is agreement that it is a suitable replacement for the iodohippurates for routine gamma camera renography [2–6] but not for the estimation of renal plasma flow [4]. As a result of these encouraging reports, a radiopharmaceutical kit has been developed for the preparation of $^{99}Tc^m$-MAG3. A limiting factor in the routine use of this kit is the short expiry of 1 h which the manufacturers recommend for the labelled product. In central radiopharmacies from which nuclear medicine departments in a number of hospitals are supplied, this short expiry is a major obstacle to the supply of $^{99}Tc^m$-MAG3 since 1 h can represent the journey time between the radiopharmacy and the nuclear medicine department. Even within one department, an expiry time of 1 h would allow only two studies to be performed from each MAG3 kit, making $^{99}Tc^m$-MAG3 expensive in comparison to other $^{99}Tc^m$ radiopharmaceuticals where many more patient doses can be prepared from a single kit. It was therefore decided to investigate the stability of $^{99}Tc^m$-MAG3 to see if under the conditions used to prepare the radiopharmaceutical in this department, the expiry time could be extended beyond the 1 h recommended.

The HPLC method used to measure the radiochemical purity of the $^{99}Tc^m$-MAG3 demonstrates the presence of six components in the radiopharmaceutical. The identity of these components is not known but it is reasonable to assume that the principal peak represents the active form of $^{99}Tc^m$-MAG3 while the others represent impurities. In the context of this study, the identity of the compound in each peak is not important since the aim is to look for changes in the composition of the radiopharmaceutical with time, and from these findings, to determine what represents a realistic expiry time for $^{99}Tc^m$-MAG3. Testing was performed immediately after preparation of $^{99}Tc^m$-MAG3, at the manufacturer's recommended expiry time of 1 h after preparation and at 6 h after preparation, this being a common expiry for $^{99}Tc^m$ radiopharmaceuticals.

In Part I of the study, when $^{99}Tc^m$-MAG3 was prepared according to the manufacturer's instructions, only one of the impurities was seen to increase significantly over a period of 6 h. This was from approximately 0.5% to 1.0% and occurred in each of the three lots of kits tested. While the increase is statistically significant, it is improbable that such a small change could be clinically significant. The method used for the preparation of $^{99}Tc^m$-MAG3 in Part I does not lend itself to routine practice in a radiopharmacy where it is necessary, in the interests of economy,

to obtain a number of doses from each kit. In Part II of the study, a means by which five individual patient doses are obtained from each MAG3 kit was investigated. To permit an administered activity of 100 MBq per patient, it was decided that single dose vials containing 175 MBq/2.5 ml would be the minimum which would allow studies to be performed up to 3 h after preparation. This was achieved by preparing the kit at a radioactive concentration of 1 GBq /4 ml and then dispensing 0.7 ml aliquots, diluted with 1.8 ml saline, into empty vials. A greater volume of reconstituted kit is required if the patient study is to be performed more than 3 h after preparation of the $^{99}Tc^{m}$-MAG3. The radiochemical purity of the single doses of $^{99}Tc^{m}$-MAG3 prepared in this way was very similar to that found in Part I except that peak 4 did not double in size over the 6 h period. It would therefore seem that diluting $^{99}Tc^{m}$-MAG3 has the effect of increasing its stability but further work is required to substantiate this. Throughout the stability studies, recovery from the HPLC column was very high showing that no impurities in the preparations were irreversibly bound to the column packing and thereby escaped detection.

The stability studies have shown that $^{99}Tc^{m}$-MAG3 prepared under the conditions described, appears to be stable for 6 h and this agrees with previously published data [11]. To confirm that this stability is borne out in practice, a clinical study was performed. Mallinckrodt's directions for the use of the MAG3 kit include the statement 'Small amounts (1–2%) of $^{99}Tc^{m}$-labelled impurities will be formed during the labelling process. As these impurities are accumulated in the liver and excreted to the gall bladder they may influence the late phase (after 30 min) of a dynamic renal study.' In the clinical study, the residual activities in the liver and gall bladder of patients who had been injected with $^{99}Tc^{m}$-MAG3 within 1 h of preparation were compared with those of patients injected 5–6 h after preparation. The technique used to measure the percentage of injected $^{99}Tc^{m}$ in the liver and gall bladder suffers from several potential sources of inaccuracy: the measurement of gall bladder depth and correction for attenuation; the assumption that the liver lies at the same depth as the gall bladder; the fact that the liver is a large organ of varying thickness; the difficulty of creating a ROI around the whole liver due to residual activity in the kidneys. Despite these sources of potential error, the object of the study was to compare the results from groups of patients. Although the results for the percentage of injected $^{99}Tc^{m}$ in the liver and gall bladder may not be accurate, they are comparable given that all images were analysed by the same technique. To ensure that the results of these clinical studies were not influenced by disparities in the renal function of the patients in the various groups, the plasma creatinine level of each patient was measured. Table 3 shows that few patients had abnormal renal function as indicated by a plasma creatinine of greater than 150 $\mu mol\ l^{-1}$ and that these patients did not predominate in any one group. The results of the clinical study confirm the findings of the *in vitro* stability study in that when $^{99}Tc^{m}$-MAG3 is administered up to 6 h after preparation, the incidence of gall bladder visualization and the degree to which $^{99}Tc^{m}$ is concentrated in the gall bladder and liver, are not increased significantly. Also, the percentage of administered $^{99}Tc^{m}$ which becomes localized in the gall bladder is very

low and it is unlikely that it will affect the result of a renal investigation if it is included in the ROI which is used to represent the right kidney.

Chromatographic analysis of urine from six patients in this study has shown that all the impurities present in $^{99}Tc^{m}$-MAG3 are excreted by the kidneys. However, the concentrations of impurities are lower in the urine than in the original injection (Table 4), showing that they are excreted less efficiently than $^{99}Tc^{m}$-MAG3.

In conclusion, this study has shown that $^{99}Tc^{m}$-MAG3, when prepared according to the methods described in this paper, is stable for 6 h.

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Original article

An investigation of factors that might influence the radiochemical purity and stability of ^{99m}Tc-MAG3

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Abstract. Technetium 99m mercaptoacetyltriglycine (^{99m}Tc-MAG3) was prepared from a commercial kit by various techniques to assess the effect of a number of variables on radiochemical purity and stability. Its radiochemical purity was determined by high performance liquid chromatography at 0 and 6 h after preparation and was found to be consistently >95%. It has been demonstrated that the radiochemical purity of ^{99m}Tc-MAG3 prepared according to the manufacturer's instructions is not influenced by the volume of ^{99m}Tc generatur eluate used, agitation, the presence of air in the reaction vial or the use of a ^{99m}Tc generator eluate with a ^{99}Tc:^{99m}Tc ratio of 16:1. A modified method of preparation in which the MAG3 kit is reconstituted with saline before addition of ^{99m}Tc-pertechnetate has been shown to yield a satisfactory product and should help to minimise the radiation dose to the fingers of radiopharmacy staff.

Key words: ^{99m}Tc-MAG3 – Radiochemical purity – Stability

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Introduction

Technetium 99m mercaptoacetyltriglycine (^{99m}Tc-MAG3) has been developed as a new radiopharmaceutical for use in gamma-camera renography (Fritzberg et al. 1986) and a number of reports have shown it to be efficacious in clinical practice (Taylor et al. 1987, 1988; Bubeck et al. 1988; Jafri et al. 1988; Al-Nahhas et al. 1988). A radiopharmaceutical kit for the preparation of ^{99m}Tc-MAG3 is available commercially and despite the manufacturer's recommended shelf-life of 1 h for the labelled product, it has been shown that its radiochemical purity can remain satisfactory for at least 6 h (Millar et al. 1990). While the instructions for reconstituting the MAG3 kit specify a number of conditions for the labelling procedure, they do not cover all variables which might affect the quality of the labelled product. This study was therefore undertaken to determine the effect of various parameters on the quality of ^{99m}Tc-MAG3 and to identify the optimal preparative conditions for high radiochemical purity and stability.

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Materials and methods

Preparation of ^{99m}Tc-MAG3. ^{99m}Tc-MAG3 was prepared from sodium pertechnetate [^{99m}Tc] injection (Fission) E.P. from a ^{99m}Tc generator (Amersham International plc, Bucks, UK) and MAG3 kits (Mallinckrodt Diagnostica BV, Petten, Netherlands) using the following nine techniques. Techniques 1–7 were performed with kits from the one lot of MAG3. After preparation, vials containing ^{99m}Tc-MAG3 were stored at room temperature.

1. ^{99m}Tc-pertechnetate (500 MBq/0.1 ml) from a generator which had been eluted within the previous 24 h was diluted to 4 ml with sodium chloride injection E.P. and injected into a MAG3 kit. The vial was placed in a boiling water bath for 10 min and then cooled for 10 min.

2. As in (1) but using ^{99m}Tc-pertechnetate (500 MBq/1.0 ml).

3. As in (1) but using ^{99m}Tc-pertechnetate (500 MBq/<1.0 ml). A 0.7 ml aliquot of the labelled product was transferred to a 10-ml, nitrogen-filled, sterile vial (Amersham, N46) and diluted to 2.5 ml with sodium chloride injection E.P. Air (10 ml) was bubbled through the solution and then the vial was placed on a rotating axle mixer for 6 h.

4. As in (1) but using ^{99m}Tc-pertechnetate (500 MBq/<1.0 ml) diluted to 10 ml with sodium chloride injection E.P.

5. As in (4) but 10 ml air was bubbled through the solution before the vial was placed in the boiling water bath.

6. As in (4) but the ^{99m}Tc-pertechnetate was from the first eluate from a new ^{99m}Tc generator.

7. A MAG3 kit was reconstituted with 9.0–9.9 ml sodium chloride injection E.P. ^{99m}Tc-pertechnetate (500 MBq/<1.0 ml) was in-

jected into the vial to give a total volume of 10 ml. Heating and cooling were performed as in (1).

8. As in (7) but using kits from five different lots of MAG3.

9. As in (8). A 1.0 ml aliquot of the labelled product was transferred to a 10-ml, nitrogen-filled, sterile vial and diluted to 2.5 ml with sodium chloride injection E.P.

Analysis of ^{99m}Tc-MAG3. The radiochemical purity of ^{99m}Tc-MAG3 was measured by high-performance liquid chromatography (HPLC) using a modification of a method provided by the manufacturer of the MAG3 kit (Mallinckrodt, personal communication). A loop-valve injector was used to inject a 20 µl sample of ^{99m}Tc-MAG3 onto a 250 × 5 mm 5-µm Hypersil ODS column (Shandon). The radiation detector was constructed from a 2 µl loop of stainless steel tubing (0.25 mm internal diameter) positioned inside a well-type sodium iodide detector which was coupled to a nucleonic counting system with outputs to a chart recorder and digital printer. The column was eluted at 1 ml/min with ethanol/0.01 *M* phosphate buffer pH 6 (5:95) for 10 min and then methanol/water (90:10) for 10 min. The counts in each peak were calculated from the output of the digital printer and expressed as a percentage of the total counts detected. The recovery of ^{99m}Tc from the HPLC column was determined by collecting the eluate and comparing its count rate with that from a 20 µl sample of the ^{99m}Tc-MAG3 under test. Radiochemical purity was measured at 0 and 6 h after labelling. ^{99m}Tc-MAG3 prepared by each procedure was tested on five occasions.

Results

A typical chromatogram is shown in Fig. 1 and demonstrates the presence of six components in a preparation of ^{99m}Tc-MAG3. Four peaks (1–4), corresponding to hydrophilic impurities, appear before the main ^{99m}Tc-MAG3 peak (5). One peak (6), corresponding to a lipophilic impurity, appears after the change of mobile phase. Peak 3 was shown to have the same retention time as pertechnetate Tc 99m.

The comparison of ^{99m}Tc-MAG3 prepared using 0.1 (technique 1) and 1.0 ml (technique 2) ^{99m}Tc generator eluate is shown in Table 1. No difference in the percentage of activity in each peak was observed. In both cases the percentage of activity in peak 4 approximately doubled over the 6-h period. The increase in peak 4 was accompanied by a corresponding decrease in peaks 5 and 6. Table 1 also shows the effect of diluting, aerating and agitating ^{99m}Tc-MAG3 (technique 3). This treatment caused an increase in the percentage activity in peak 3 but eliminated the doubling of peak 4 over the 6-h period.

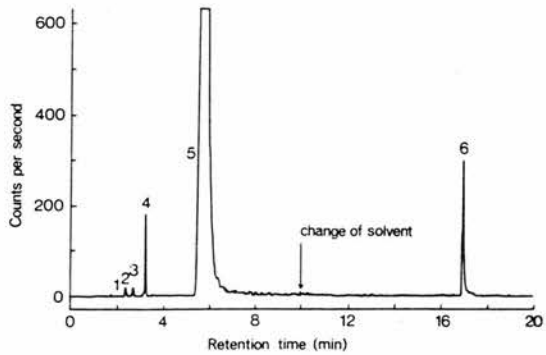

Fig. 1. High performance liquid chromatogram of mercaptoacetyltriglycine Tc 99m (^{99m}Tc-MAG3)

All four techniques (4–7) used to prepare ^{99m}Tc-MAG3 at a radioactive concentration of 500 MBq/10 ml resulted in similar percentages of activity in each peak (Table 2). As before, a doubling of peak 4 over the 6-h period was observed.

Table 3 shows the comparison of undiluted (technique 8) and diluted (technique 9) ^{99m}Tc-MAG3 prepared from five lots of kits. As before, a doubling of peak 4 in 6 h is seen in the undiluted material, while this effect is eliminated in the diluted preparations.

In all experiments, the recovery of ^{99m}Tc from the HPLC column was approximately 100%.

Table 1. Results of high-performance liquid chromatographic analysis of ^{99m}Tc-MAG3 prepared at 500 MBq/4 ml

Conditions for preparation	Time after preparation (h)	Percentage of the recovered activity in each peak						Percentage recovery from column
		1	2	3	4	5	6	
0.1 ml eluate	0	0.2±0.2	0.1±0.1	0.3±0.1	0.6±0.1	96.4±0.4	2.4±0.5	99.0 ±2.3
	6	0.2±0.1	0.2±0.1	0.4±0.2	1.3±0.3	96.2±0.2	1.8±0.2	99.6 ±2.3
1.0 ml eluate	0	0.1±0.0	0.1±0.1	0.2±0.1	0.6±0.1	96.7±0.3	2.3±0.2	101.5±1.2
	6	0.1±0.0	0.1±0.0	0.5±0.2	1.2±0.2	96.2±0.2	1.9±0.1	101.5±1.8
<1.0 ml eluate plus dilution, air and agitation	0	0.2±0.1	0.3±0.1	0.8±0.5	0.4±0.1	95.9±0.7	2.4±0.3	101.0±2.9
	6	0.1±0.1	0.3±0.1	1.0±0.5	0.5±0.2	95.8±0.9	2.3±0.3	100.6±2.4

MAG3, mercaptoacetyltriglycine
Each value is the mean ± SD of five results

Table 2. Results of high-performance liquid chromatographic analysis of ^{99m}Tc-MAG3 prepared at 500 MBq/10 ml

Conditions for preparation	Time after preparation (h)	Percentage of the recovered activity in each peak						Percentage recovery from column
		1	2	3	4	5	6	
<1.0 ml eluate	0	0.1±0.1	0.1±0.0	0.1±0.0	0.5±0.1	96.4±0.5	2.8±0.4	99.0±3.0
	6	0.1±0.0	0.1±0.1	0.3±0.0	0.9±0.1	96.2±0.5	2.5±0.2	99.9±3.1
Air before boiling	0	0.1±0.0	0.1±0.1	0.1±0.1	0.4±0.1	96.8±0.5	2.5±0.4	99.8±3.5
	6	0.0±0.0	0.1±0.1	0.2±0.1	0.9±0.1	96.3±0.4	2.5±0.3	99.3±2.1
Eluate from new generator	0	0.3±0.1	0.1±0.0	0.1±0.1	0.3±0.1	96.9±0.1	2.3±0.2	100.1±1.3
	6	0.1±0.0	0.1±0.0	0.2±0.1	0.8±0.1	96.7±0.3	2.1±0.2	99.2±0.6
Saline before ^{99m}Tc-pertechnetate	0	0.1±0.0	0.1±0.0	0.1±0.0	0.3±0.0	97.3±0.1	2.1±0.1	98.6±3.3
	6	0.0±0.0	0.0±0.1	0.2±0.1	0.8±0.1	96.8±0.2	2.1±0.1	101.9±1.5

MAG3, mercaptoacetyltriglycine
Each value is the mean ± SD of five results

Table 3. Results of the comparison of undiluted and dilute ^{99m}Tc-MAG3

Conditions for preparation	Time after preparation (h)	Percentage of the recovered activity in each peak						Percentage recovery from column
		1	2	3	4	5	6	
Undiluted	0	0.1±0.1	0.1±0.1	0.1±0.1	0.3±0.1	97.5±0.3	1.9±0.2	96.8±2.2
	6	0.1±0.1	0.1±0.1	0.2±0.1	0.8±0.1	97.1±0.5	1.7±0.4	98.0±1.2
Diluted	0	0.1±0.0	0.2±0.1	0.1±0.0	0.3±0.1	97.5±0.4	1.8±0.4	98.4±1.7
	6	0.1±0.0	0.2±0.1	0.2±0.1	0.3±0.2	97.1±0.4	2.1±0.3	100.4±1.3

MAG3, mercaptoacetyltriglycine
Each value is the mean ± SD of five results

Discussion

^{99m}Tc-MAG3 is an important new radiopharmaceutical for the assessment of renal function. Its preparation is straightforward, using a radiopharmaceutical kit which is available commercially. The manufacturers of this kit specify certain conditions for the preparation of ^{99m}Tc-MAG3 but do not provide information on all the variables which might affect its quality. This investigation was therefore undertaken to establish how factors such as the presence of air, concentration of ^{99}Tc and volume of generator eluate influence the radiochemical purity of ^{99m}Tc-MAG3.

The instructions for preparing ^{99m}Tc-MAG3 specify that the kit should be reconstituted with 4 ml pertechnetate solution, 0.1–1.0 ml of which is ^{99m}Tc generator eluate. It could be assumed, therefore, that if more than 1.0 ml generator eluate is used, an inferior quality product is obtained. Conversely, it might be that the smaller the volume of eluate used, the superior the product obtained. ^{99m}Tc-MAG3 prepared using the volumes of eluate at either end of the recommended range (techniques 1 and 2) were compared to determine whether the lower volume resulted in a product with a higher radiochemical purity. This was not shown to be so. Within the recommended range of eluate volumes, ^{99m}Tc-MAG3 of consistent radiochemical purity was obtained.

In radiopharmacies which supply a number of nuclear medicine departments, a policy of preparing individual patient doses of radiopharmaceuticals is often adopted. Subdividing kits of ^{99m}Tc radiopharmaceuticals after preparation is therefore a common practice. This process of subdivision can be accompanied by dilution, during which air can be introduced into the vial. In some situations, the individual patient doses are transported some considerable distance to the nuclear medicine department. During transport, the radiopharmaceuticals are invariably subjected to agitation. The effect of dilution, aeration and agitation of ^{99m}Tc-MAG3 was therefore studied (technique 3). The percentage of activity in peak 3 approximately doubled following this treatment and since peak 3 was found to have the same retention time as ^{99m}Tc-pertechnetate, this would be consistent with an increased pertechnetate content caused by oxidation. Despite this increased level of impurity, the

radiochemical purity remained satisfactory at greater than 95%. Unlike the previous experiment, the percentage activity in peak 4 did not double over the 6 h of the study. This agrees with our previous findings (Millar et al. 1990).

At this point in the study, the manufacturer of the MAG3 kit issued new recommendations for preparing ^{99m}Tc-MAG3. The total volume of pertechnetate solution to be added to the kit was increased from 4 ml to 10 ml, up to 3 ml of which could be ^{99m}Tc generator eluate. Under these revised conditions, the manufacturer's recommended shelf-life for the labelled product was increased from 1 h to 4 h. The effect of this revised procedure was investigated (technique 4), but the volume of ^{99m}Tc generator eluate was maintained at <1 ml as in the previous experiments. The radiochemical purity and stability of the ^{99m}Tc-MAG3 prepared by this technique was comparable to that observed in the previous experiments.

In an earlier experiment, the effect of bubbling air through the labelled product was investigated. It was therefore decided also to study the effect of introducing air before the boiling step of the labelling procedure (technique 5) since it is conceivable that this could occur during the routine preparation of ^{99m}Tc-MAG3. It was found, however, that this did not affect radiochemical purity.

On certain days of the week, principally Mondays, it is not possible to avoid preparing ^{99m}Tc radiopharmaceuticals with ^{99m}Tc-pertechnetate obtained from a generator which has remained uneluted for a few days. Under these conditions, the level of ^{99}Tc in the eluate becomes high, and it is theoretically possible that this can be deleterious to the quality of radiopharmaceuticals prepared from it. The effect of ^{99}Tc was therefore investigated by preparing ^{99m}Tc-MAG3 using the first eluate from a new ^{99m}Tc generator (technique 6). From the time between the last separation of Mo and Tc performed by the generator manufacturer and the time of the first elution it has been possible to calculate the ratio of ^{99}Tc: ^{99m}Tc as 16 (Bauer and Pabst 1982). With this excess of ^{99}Tc in the generator eluate no effect was observed on the radiochemical purity of the ^{99m}Tc-MAG3 prepared from it.

During the preparation of ^{99m}Tc-MAG3 according to the manufacturer's revised instructions, the withdrawal of ^{99m}Tc-pertechnetate (500 MBq/10 ml) into a syringe followed by the injection of the solution into the MAG3 kit is a slow process which will result in a radiation dose to the fingers of the radiopharmacy staff. In an attempt to minimise handling of the ^{99m}Tc, a technique in which the contents of the MAG3 kit are dissolved in saline and then the ^{99m}Tc-pertechnetate is added in a volume of less than 1 ml was investigated (technique 7). In this technique, the small volume of ^{99m}Tc-pertechnetate can be transferred quickly to the kit, thus minimising handling time and therefore finger dose. The radiochemical purity of the ^{99m}Tc-MAG3 prepared by this modified technique was comparable to that obtained with the manufacturer's recommended technique. This modified technique should therefore help to limit finger radiation dose during the preparation of ^{99m}Tc-MAG3.

Clinical experience with ^{99m}Tc-MAG3 supplied from this radiopharmacy has shown that adequate renogram curves can be obtained with 20 MBq. The feasibility of dispensing 10×50 MBq doses from each MAG3 kit was therefore investigated. ^{99m}Tc-MAG3 was prepared as 500 MBq/10 ml (technique 8), and 1.0 ml aliquots were diluted to give individual patient doses of 50 MBq/2.5 ml (technique 9). The radiochemical purities of the undiluted and diluted ^{99m}Tc-MAG3 solutions were compared and found to be very similar. As had been observed in previous experiments, the undiluted material showed a doubling of the impurity in peak 4 over 6 h while the diluted material did not.

When analysing radiopharmaceuticals by HPLC, some of the radionuclide injected into the chromatograph may become irreversibly bound to the column packing. This adsorbed radionuclide does not appear as a peak on the chromatogram and therefore remains undetected. If this undetected material is an impurity, then a falsely high result for radiochemical purity is obtained. It is important therefore to measure the recovery of the radionuclide from the HPLC column. In all parts of this study the recovery from the HPLC column was approximately 100%, showing that there are no major impurities in ^{99m}Tc-MAG3 which remain undetected.

In conclusion, this study has demonstrated the following. The radiochemical purity of ^{99m}Tc-MAG3 prepared according to the manufacturer's instructions is not influenced by the volume of ^{99m}Tc generator eluate, agitation, the presence of air in the reaction vial or the use of a ^{99m}Tc generator eluate with a ^{99}Tc: ^{99m}Tc ratio of 16:1. A modified method for reconstituting the MAG3 kit reduces handling of the ^{99m}Tc during preparation of ^{99m}Tc-MAG3 and should therefore help to minimise the radiation dose to the fingers of radiopharmacy staff.

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A routine method for using sodium iodide to stabilize sodium pertechnetate [$^{99}Tc^m$] dispensed for the preparation of $^{99}Tc^m$-exametazime

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Summary

When preparing $^{99}Tc^m$-exametazime, it is important to use sodium $^{99}Tc^m$-pertechnetate that is less than 2 h old. The addition of sodium iodide (NaI) to $^{99}Tc^m$-pertechnetate is known to extend this time to 6 h. This paper describes a technique for implementing this in routine practice. Sterile kits consisting of 440 μg NaI and 1.0 ml sodium chloride injection in a vial with a nitrogen atmosphere were prepared and stored at room temperature, 4°C and −22°C. Titrimetric analysis of iodide showed that under each storage condition, kits were stable for 8 weeks. To determine the effectiveness of the kits, the radiochemical purity (RCP) of $^{99}Tc^m$-exametazime was measured by high-performance liquid chromatography (HPLC). The validity of this technique was determined by simultaneous analysis with the conventional thin-layer/paper chromatography (TLC/PC) technique on 24 occasions, over a range of RCP (94.5–54.6%). Radiochemical purities measured by HPLC and TLC/PC were 81.2 ± 10.2 and 81.5 ± 10.5%, respectively, and did not differ significantly ($P>0.30$). The correlation between the techniques was high ($r=0.98$). $^{99}Tc^m$-exametazime was prepared using 1 h-old $^{99}Tc^m$-pertechnetate, 6 h-old $^{99}Tc^m$-pertechnetate and 6 h-old $^{99}Tc^m$-pertechnetate dispensed in a NaI kit. At the recommended expiry time for this radiopharmaceutical, i.e. 30 min after preparation, RCPs were found to be 88.4 ± 2.4, 80.9 ± 2.0 and 89.3 ± 3.0%, respectively ($n=5$ for each technique). At this time, the RCPs of the products prepared from 1 h-old $^{99}Tc^m$-pertechnetate and 6 h-old $^{99}Tc^m$-pertechnetate were significantly different ($P<0.01$) while the RCPs of the products prepared from 1 h-old $^{99}Tc^m$-pertechnetate and 6 h-old $^{99}Tc^m$-pertechnetate + NaI did not differ significantly ($P>0.50$). In conclusion, the use of a NaI kit is a satisfactory means of increasing the stability of $^{99}Tc^m$-pertechnetate that is to be used in the preparation of $^{99}Tc^m$-exametazime.

Introduction

$^{99}Tc^m$-exametazime is a radiopharmaceutical that is used for brain imaging [1] and radiolabelling of leucocytes [2]. It is prepared using a commercially available freeze-dried kit. A major limitation in the use of this kit is its expiry time of 30 min after reconstitution. A consequence of this short expiry is that the $^{99}Tc^m$-exametazime must be prepared immediately before it is to be used. This may mean that reconstitution of the kit cannot be undertaken in the radiopharmacy but must be carried out in the department in which the $^{99}Tc^m$-exametazime is to be used. The manufacturer also recommends that the kit is reconstituted with sodium pertechnetate [$^{99}Tc^m$] injection BP ($^{99}Tc^m$-pertechnetate) obtained from a generator eluate that is less than 2 h old. This can represent a logistical problem if the nuclear medicine department is in a different institution to the radiopharmacy or if the $^{99}Tc^m$-exametazime is to be administered more than 2 h after the time at which the $^{99}Tc^m$ generator is eluted routinely. Bayne *et al.* [3] have reported that the addition of sodium iodide to the generator eluate overcomes this problem and extends the shelf-life of $^{99}Tc^m$-pertechnetate from 2 to 6 h. This paper describes a method by which this procedure can be introduced into routine use.

Materials and methods

Preparation of sodium iodide kits

Sodium iodide BP (Thornton Ross) was dissolved in sodium chloride injection BP to give a solution of

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concentration 440 μg ml^{-1}. Sodium iodide kits were prepared by filtering 1.0 ml aliquots of this solution through a sterile 220 nm filter into weighed, sterile 10 ml nitrogen-filled vials (Code N46, Amersham International plc). The 220 nm filter was primed with sodium iodide solution before use to avoid adsorption and dead space problems. Each vial was reweighed and the weight of solution calculated. From these weights, the sodium iodide content of each kit was calculated. Five batches of kits were prepared. From each batch, 10 vials were stored at room temperature, 10 in a refrigerator at 4°C and 10 in a freezer at −22°C.

Assay of iodide content of kits

At 0, 1, 2, 4 and 8 weeks after preparation, the contents of two kits that had been kept under each storage condition were assayed by the following technique to determine iodide content. This technique is a miniaturization of the British Pharmacopoeia (BP) assay for sodium iodide [4]. One vial from each pair was centrifuged to bring all the solution to the bottom of the vial. The rubber cap was removed from the vial. Hydrochloric acid, 1.2 ml, was pipetted into the vial which was then reweighed. Approximately 0.1 ml 3 mM potassium iodate solution was added to the vial to develop a yellow colour and the vial was reweighed. Amaranth solution (1/100 strength), 0.1 ml, was added to the vial which was reweighed. Potassium iodate solution, 3 mM, was added until the colour of the solution changed from pink to pale yellow. The vial was reweighed. From the total weight of 3 mM potassium iodate solution added, the iodide content of the vial was calculated (each ml of 3 mM potassium iodate is equivalent to 899 μg of sodium iodide). One drop of starch mucilage BP was injected into the other vial of the pair. A blue colour developed if >1% of the iodine was present in the form of I_2.

Preparation of $^{99}Tc^m$-exametazime

$^{99}Tc^m$-pertechnetate solutions for use in the preparation of $^{99}Tc^m$-exametazime were obtained from a $^{99}Tc^m$ generator (Code MCC20, Amersham International plc) that had been eluted within the previous 24 h and were dispensed immediately after elution of the generator. $^{99}Tc^m$-exametazime injections were prepared by injecting 5 ml of one of the following solutions into a Ceretec kit (Code N109, Amersham International plc):

1. *1 h $^{99}Tc^m$-pertechnetate*: $^{99}Tc^m$ generator eluate was diluted to a radioactive concentration of 1.3 GBq per 5.5 ml, injected into a sterile 10 ml nitrogen-filled vial and used 1 h later.
2. *6 h $^{99}Tc^m$-pertechnetate*: As in 1, but at a radioactive concentration of 2.6 GBq per 5.5 ml and used 6 h later by which time the radioactive concentration had fallen to 1.3 GBq per 5.5 ml.
3. *6 h $^{99}Tc^m$-pertechnetate + NaI*: $^{99}Tc^m$ generator eluate was diluted to a radioactive concentration of 2.6 GBq per 5.5 ml in a sodium iodide kit and used 6 h later by which time the radioactive concentration had fallen to 1.3 GBq per 5.5 ml.

Five $^{99}Tc^m$-exametazime injections were prepared by each of the three techniques. $^{99}Tc^m$-exametazime injections were stored at room temperature.

Analysis of $^{99}Tc^m$-exametazime

The radiochemical purity of each $^{99}Tc^m$-exametazime injection was measured at 2, 30, 60 and 120 min after preparation. Radiochemical purity was measured by high-performance liquid chromatography (HPLC) using a modification of the method of Neirinckx *et al.* [1]. A loop-valve injector was used to inject a 20 μl sample of $^{99}Tc^m$-exametazime onto a 150 × 4.6 mm 10 μm PRP-1 column (Hamilton) which was fitted with a 25 × 3 mm PRP-1 guard column. The radiation detector was constructed from a 40 mm length of stainless steel column outlet tubing (0.25 mm internal diameter) placed across the face of a sodium iodide scintillation detector. The output from the detector was taken to a Specmate preamplifier/amplifier (Canberra Nuclear Data) and from there to an Accuspec PC-based multichannel analyser (Canberra Nuclear Data) which was operated in multichannel scaling mode. The column was eluted with 20 mM phosphate buffer, pH 7.4. A linear gradient of 0–25% tetrahydrofuran over 6 min was started when the sample was injected. The chromatogram was acquired in the multichannel analyser. A region of interest was placed around each peak on the chromatogram and the counts in each region were recorded. The radiochemical purity was calculated by expressing the counts in the peak corresponding to the primary $^{99}Tc^m$-exametazime complex as a percentage of the total counts in the chromatogram.

The recovery of $^{99}Tc^m$ from the HPLC column was determined by collecting the eluate and comparing its count rate with that from a 20 μl sample of the $^{99}Tc^m$-exametazime being tested.

To validate the HPLC technique as a satisfactory method for determining radiochemical purity, the first two samples of $^{99}Tc^m$-exametazime prepared from each of the three solutions of $^{99}Tc^m$-pertechnetate were analysed by HPLC and the standard three-system thin-layer/paper chromatography (TLC/PC) technique recommended by Amersham [1,5].

Results

For each storage condition, the iodide content of the kits remained >96% throughout the 8 weeks of the study. No kit was found to contain >1% of the iodine in the

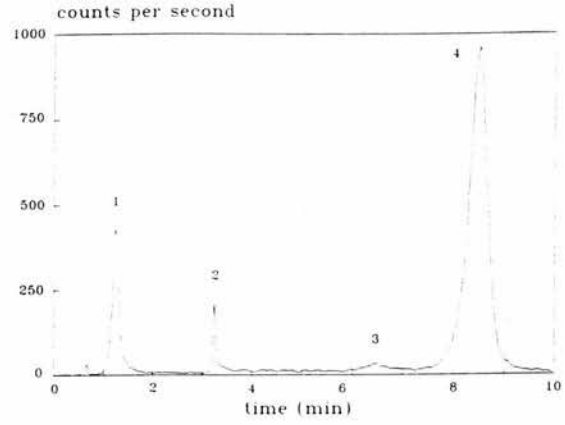

Fig. 1. High-performance liquid chromatogram of $^{99}Tc^{m}$-exametazime.

form of I_2. A typical chromatogram of $^{99}Tc^{m}$-exametazime is shown in Fig. 1. The chromatogram contains four major peaks. Peaks 1, 2 and 4 correspond to $^{99}Tc^{m}$-pertechnetate, secondary $^{99}Tc^{m}$-exametazime complex and primary $^{99}Tc^{m}$-exametazime complex, respectively. The identity of peak 3 remains unknown. Peak 3 was found to contain approximately 3.5% of the total activity and this remained unchanged over the 2 h of the study. The activities in peaks 1 and 2 increased with time.

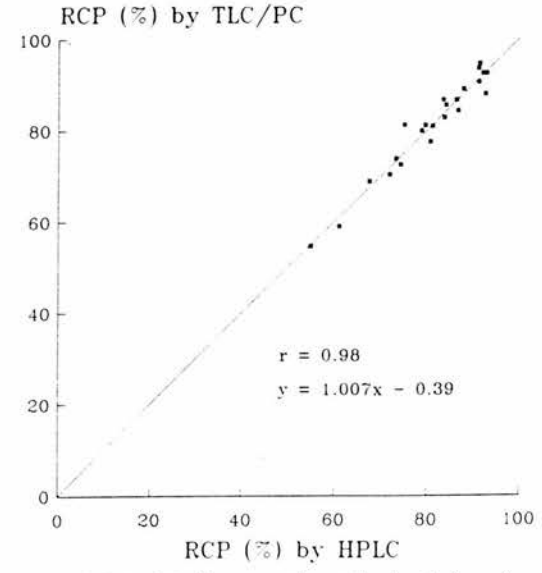

Fig. 2. Relationship between the radiochemical purity of $^{99}Tc^{m}$-exametazime as measured by HPLC and TLC/PC.

Table 1. The effect of three preparations of $^{99}Tc^{m}$-pertechnetate on the radiochemical purity of $^{99}Tc^{m}$-exametazime.

Time after preparation (min)	*Radiochemical purity (%)*		
	1 h $^{99}Tc^{m}$	*6 h $^{99}Tc^{m}$*	*6 h $^{99}Tc^{m}$ + NaI*
2	92.6 ± 1.3	93.2 ± 1.7	93.1 ± 2.0
30	88.4 ± 2.4	80.9 ± 2.0	89.3 ± 3.0
60	83.2 ± 4.1	70.7 ± 3.2	86.1 ± 4.6
120	74.7 ± 5.0	56.5 ± 4.0	81.2 ± 6.4

Each value is the mean ± S.D. of five results.

For the six preparations of $^{99}Tc^{m}$-exametazime analysed by both techniques, the mean ±standard deviation radiochemical purity determined by TLC/PC was 81.5 ± 10.5% and by HPLC was 81.2 ± 10.2%. When compared using a paired Student's *t*-test, these results were not found to be significantly different ($P>0.30$). The excellent correlation ($r=0.98$) between the two techniques is shown in Fig. 2. The mean ±standard deviation recovery

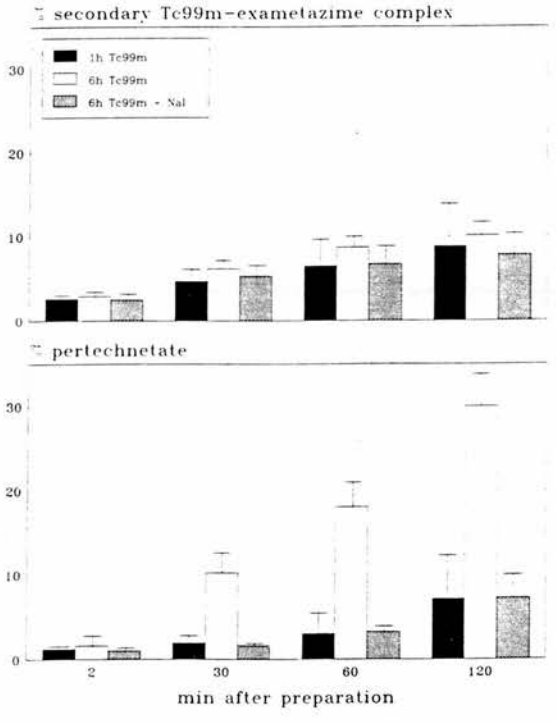

Fig. 3. Increase in the levels of secondary $^{99}Tc^{m}$-exametazime complex and $^{99}Tc^{m}$-pertechnetate impurities in $^{99}Tc^{m}$-exametazime.

of $^{99}Tc^m$ from the HPLC column for the 60 analyses performed was 99.8 ± 2.0%.

The effects of the three $^{99}Tc^m$-pertechnetate solutions on the radiochemical purity of $^{99}Tc^m$-exametazime are shown in Table 1. Using Amersham's specification of 80% being the lowest satisfactory radiochemical purity, $^{99}Tc^m$-exametazime prepared with 1 h-old or 6 h-old $^{99}Tc^m$-pertechnetate containing sodium iodide was satisfactory up to 1 h after preparation. The mean radiochemical purity of $^{99}Tc^m$-exametazime prepared with 6 h-old $^{99}Tc^m$-pertechnetate reached the 80% limit 30 min after preparation with two of the five results being <80%. Statistical analysis of the 30 min results using Dunnet's test for the comparison of group means to a control group mean [6], demonstrated that the radiochemical purities of the products prepared with 1 h-old $^{99}Tc^m$-pertechnetate and 6 h-old $^{99}Tc^m$-pertechnetate were significantly different ($P<0.01$), but that the radiochemical purities of the products prepared with 1 h-old $^{99}Tc^m$-pertechnetate and 6 h-old $^{99}Tc^m$-pertechnetate containing sodium iodide did not differ significantly ($P>0.50$).

Figure 3 shows the changes in the levels of $^{99}Tc^m$-pertechnetate and secondary $^{99}Tc^m$-exametazime impurities with time. With each method of preparation, the increase in the level of secondary $^{99}Tc^m$-exametazime complex is similar, rising to approximately 10% after 2 h. The level of the $^{99}Tc^m$-pertechnetate impurity increases much more rapidly in the $^{99}Tc^m$-exametazime prepared from the 6 h-old $^{99}Tc^m$-pertechnetate than in the other two.

Discussion

$^{99}Tc^m$-exametazime is an important new radiopharmaceutical which unfortunately suffers from instability. After preparation, the primary $^{99}Tc^m$-exametazime complex, which is the active radiopharmaceutical, converts slowly to an inactive secondary complex and undergoes breakdown to release $^{99}Tc^m$ in the form of $^{99}Tc^m$-pertechnetate [1]. To minimize this breakdown, stringent conditions are specified for the preparation of $^{99}Tc^m$-exametazime: the kit must be reconstituted with $^{99}Tc^m$ which was eluted less than 2 h previously; the generator from which the $^{99}Tc^m$ is obtained must have been eluted within the previous 24 h; the $^{99}Tc^m$-exametazime must be used within 30 min of reconstitution of the kit. Complying with the requirement that the $^{99}Tc^m$ must be obtained from a generator which has been eluted within the previous 24 h should not be a problem since, in most radiopharmacies, $^{99}Tc^m$ generators are eluted at least once daily. It is necessary, however, to avoid the use of $^{99}Tc^m$ from the first elution of a newly delivered generator or from a generator eluted on a Monday morning if it has lain uneluted over the weekend. The requirement that the $^{99}Tc^m$-exametazime must be used within 30 min of preparation can be achieved by reconstituting the kit immediately prior to use. This may mean that a vial of sodium pertechnetate [$^{99}Tc^m$] injection and an exametazime kit are issued from the radiopharmacy rather than the labelled product and that reconstitution is performed in the department in which the $^{99}Tc^m$-exametazime is to be used. The requirement that the $^{99}Tc^m$-pertechnetate used to prepare $^{99}Tc^m$-exametazime must be less than 2 h old is much more difficult to accommodate. It can be inconvenient if the $^{99}Tc^m$-exametazime is to be used more than 2 h after a routine radiopharmacy dispensing session since an additional generator elution will be required. This may reduce the generator yield at the following dispensing session. Meeting the 2 h criterion may be impossible if radiopharmaceuticals are supplied from a central radiopharmacy distant from the department in which the exametazime kit is to be reconstituted and used. Bayne *et al.* [3] have shown that the 2 h restriction can be extended to at least 6 h by the addition of sodium iodide to $^{99}Tc^m$-pertechnetate that is to be used for preparation of $^{99}Tc^m$-exametazime but have not described a technique by which this finding can be put into routine radiopharmacy practice.

As a convenient means of using this stabilization technique routinely, it was decided to investigate a kit approach by preparing batches of vials containing sodium iodide. Ideally these kits of sodium iodide would be stable for several weeks and on each occasion that $^{99}Tc^m$-pertechnetate was required for the preparation of $^{99}Tc^m$-exametazime, it would be dispensed into a kit within 30 min of generator elution.

Most commercially manufactured radiopharmaceutical kits are formulated as freeze-dried products. Since freeze-drying is not a technique available to most hospital radiopharmacies, it was decided to investigate the suitability of a kit containing a solution of sodium iodide. Bayne *et al.* [3] demonstrated that 400 μg sodium iodide was adequate for stabilization of the maximum activity (1.11 GBq) of $^{99}Tc^m$ recommended for the reconstitution of an exametazime kit. It was therefore decided to prepare kits containing 440 μg sodium iodide in 1.0 ml sodium chloride injection BP. The principle behind this technique is as follows. $^{99}Tc^m$ generator eluate, diluted to have a radioactive concentration of 1.2 GBq per 4.5 ml at the time when the exametazime kit will be reconstituted, is injected into a sodium iodide kit. The kit then contains 1.2 GBq $^{99}Tc^m$ and 440 μg sodium iodide in 5.5 ml. Amersham International recommend that their exametazime kit is reconstituted to a volume of 5 ml. Using 5 ml of the $^{99}Tc^m$ sodium iodide solution prepared as described above results in the exametazime kit being reconstituted with 1.1 GBq $^{99}Tc^m$ containing 400 μg sodium iodide.

Aqueous solutions of sodium iodide gradually become

coloured on exposure to light and air due to the liberation of iodine [7]. Vials containing a nitrogen atmosphere were therefore used in the preparation of the sodium iodide kits which were then stored in the dark. Sodium chloride injection BP which has a slightly acidic pH is not the ideal solvent for sodium iodide which is most stable under the slightly alkaline conditions of pH 8–9.5 [8]. However, pH adjustment of the solution used to prepare the sodium iodide kits was considered inadvisable since pH is known to be important in the labelling of exametazime. Reconstitution with a pH-adjusted solution might therefore upset the labelling reaction and result in low radiochemical purity. In view of these potential problems, the first step in determining the feasibility of the sodium iodide kit concept was to measure the stability of the kits and determine if it is influenced by the conditions under which they are stored. To measure stability, a modification of the 1968 BP method for determination of the iodide content of sodium iodide was used. The modification was necessary due to the small volume of solution in each kit and the small amount of sodium iodide present. The 1968 method was preferred to the methods introduced in more recent editions of the BP since it was more readily miniaturized. The analytical technique depends upon hydrochloric acid converting iodide to iodine which is then titrated with potassium iodate solution. The level of iodine present in the kit as a result of degradation of iodide would not therefore be revealed by this titrimetric analysis. A separate test for iodine was therefore performed. Regardless of whether the kits were stored at room temperature, in a refrigerator or in a freezer, they were shown to be stable for 8 weeks. Since the kits are prepared by an aseptic technique, which always carries a risk of bacterial contamination, it was decided to store the kits in a refrigerator since this is convenient and will inhibit the growth of any contamination introduced during preparation. It was also decided to err on the side of safety and give the kits an expiry time of 4 weeks.

Having established the stability of the sodium iodide kits, a set of experiments was performed to demonstrate their effectiveness at prolonging the shelf-life of $^{99}Tc^m$-pertechnetate used for the preparation of $^{99}Tc^m$-exametazime. To do this, it was necessary to measure the radiochemical purity of $^{99}Tc^m$-exametazime. This was performed by HPLC rather than the more conventional TLC/PC technique recommended by Amersham International. High-performance liquid chromatography was chosen for a number of reasons. A measurement only takes 12 min compared to the 60 min required for TLC/PC. For a radiopharmaceutical with a shelf-life of only 30 min, an analytical technique which takes 60 min to perform seems inappropriate. High-performance liquid chromatography is also a specific technique in that it displays a peak for the primary $^{99}Tc^m$-exametazime complex. In contrast, the TLC/PC technique is non-specific in that it relies on measuring the levels of impurities in the radiopharmaceutical and assuming that the remainder of the product is primary complex. To compare HPLC with TLC/PC, radiochemical purity in the first six experiments was determined by both techniques. Figure 2 shows that the correlation between the techniques is excellent (r=0.98) and the results obtained were not found to differ significantly. The excellent recovery of $^{99}Tc^m$ from the HPLC column demonstrates that no significant radiochemical impurities in $^{99}Tc^m$-exametazime become adsorbed onto the column packing material and remain undetected. Therefore, HPLC was considered to be a suitable technique for determining the radiochemical purity of $^{99}Tc^m$-exametazime.

Having assigned a 4-week expiry to the sodium iodide kits, it was decided that the most challenging test of their effect on the radiochemical purity of $^{99}Tc^m$-exametazime would be to use them 4 weeks after preparation. In the comparison of the $^{99}Tc^m$-exametazime prepared using the three $^{99}Tc^m$-pertechnetate solutions, the radiochemical purities of the products prepared using the 1 h-old $^{99}Tc^m$-pertechnetate and the 6 h-old $^{99}Tc^m$-pertechnetate with sodium iodide were similar over the 2 h of the study and remained satisfactory, i.e. higher than the 80% limit, up to 1 h after preparation. In contrast, the radiochemical purities of two preparations of $^{99}Tc^m$-exametazime prepared with 6 h-old $^{99}Tc^m$-pertechnetate were <80% within 30 min. These results demonstrate the effectiveness of the sodium iodide kits in prolonging the shelf-life of $^{99}Tc^m$-pertechnetate which is to be used in the preparation of $^{99}Tc^m$-exametazime.

The results shown in Fig. 3 demonstrate that the rise in the level of secondary $^{99}Tc^m$-exametazime complex is similar in all three preparations and that free $^{99}Tc^m$-pertechnetate is the impurity responsible for the rapid fall in the radiochemical purity of the $^{99}Tc^m$-exametazime prepared from 6 h-old $^{99}Tc^m$-pertechnetate.

In conclusion, the sodium iodide kits described in this paper are suitable for use in routine radiopharmacy practice as a means of extending the shelf-life of $^{99}Tc^m$-pertechnetate which is to be used in the preparation of $^{99}Tc^m$-exametazime.

Acknowledgements

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Effect of source and age of sodium pertechnetate Tc 99m on radiochemical purity of technetium Tc 99m exametazime

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Abstract: The radiochemical purities of technetium Tc 99m exametazime prepared with one-hour-old or six-hour-old sodium pertechnetate Tc 99m from two manufacturers' generators were compared.

Eluates from each manufacturer's generators were diluted immediately to provide two solutions of sodium pertechnetate Tc 99m. For the one-hour-old solution, eluate was diluted with 0.9% sodium chloride injection to a concentration of radioactivity of 38 mCi in 5.5 mL and used one hour later. For the six-hour-old solution, eluate was diluted to 65 mCi in 5.5 mL and used six hours later. Technetium Tc 99m exametazime was prepared by injecting 5.0 mL of one of the solutions into an exametazime kit to provide 30 mCi of technetium Tc 99m in 5.0 mL. At 2, 30, and 60 minutes after reconstitution of each kit, the radiochemical purity was measured by high-performance liquid chromatography with radiation detection.

At two minutes, all the preparations retained high radiochemical purities. However, at 30 and 60 minutes, the radiochemical purities of technetium Tc 99m exametazime prepared with six-hour-old sodium pertechnetate Tc 99m were significantly lower than those of technetium Tc 99m exametazime prepared with one-hour-old sodium pertechnetate Tc 99m. Similar results were found for each manufacturer's generators.

The radiochemical purity of technetium Tc 99m exametazime was affected by the age of the sodium pertechnetate Tc 99m from which it was prepared but not by the generator from which the sodium pertechnetate Tc 99m was obtained.

Index terms: Drugs; Injections; Manufacturers; Purity; Roentgenographic agents; Sodium pertechnetate Tc 99m; Stability; Technetium Tc 99m exametazime

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Technetium Tc 99m exametazime is a radiopharmaceutical that is used in imaging cerebral blood flow[1] and in labeling autologous leukocytes for the detection of sites of inflammation.[2] Technetium Tc 99m exametazime is prepared by injecting a solution of sodium pertechnetate Tc 99m into an exametazime kit. The manufacturer of this kit makes the following recommendations about its reconstitution: (1) The sodium pertechnetate Tc 99m must have been eluted from a technetium Tc 99m generator less than two hours before being used to reconstitute the kit, (2) the previous elution of the technetium Tc 99m generator must have occurred within the preceding 24 hours, (3) not more than 1.1 gigabecquerels (GBq) (30 mCi) of technetium Tc 99m must be injected into the kit, and (4) the radiopharmaceutical must be administered to the patient within 30 minutes after preparation.[3] Failure to comply with any one of these recommendations may result in administration of technetium Tc 99m exametazime of low radiochemical purity.

To comply with recommendation 4, the exametazime kit must be reconstituted not more than 30 minutes before the radiopharmaceutical is required. When the radiopharmaceutical is to be used in a department that is not located close to the pharmacy, the sodium pertechnetate Tc 99m and the exametazime kit must be supplied separately. Reconstitution is then performed in the department where the radiopharmaceutical is to be administered. In such circumstances, however, it may not be possible to reconstitute the kit within two hours of elution. Complying with the two-hour restriction on the age of sodium pertechnetate Tc 99m can therefore present a real problem. In an examination of this problem, Ponto[4] published data showing that when five-hour-old sodium pertechnetate Tc 99m eluted from a Medi-Physics or Mallinckrodt generator was used, technetium Tc 99m exametazime of high radiochemical purity was obtained immediately after preparation.

During previous work in my department, high radiochemical purity was observed immediately after reconstitution when technetium Tc 99m exametazime was prepared by using sodium pertechnetate Tc 99m from a generator manufactured by Amersham International.[5] However, when six-hour-old sodium pertechnetate Tc

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99m was used, the radiochemical purity of the technetium Tc 99m exametazime 30 minutes after preparation was considerably lower than when one-hour-old sodium pertechnetate Tc 99m was used.

In the study documented here, I attempted to establish (1) whether the phenomenon reported by Ponto[4] was a feature of the sodium pertechnetate Tc 99m obtained from the generators used in his study and (2) whether the reported insignificance of eluate age is valid at the 30-minute expiration time of technetium Tc 99m exametazime.

Methods

The technetium Tc 99m generators used were four UltraTechneKow 8.5-GBq (230-mCi) generators (product code DRN 4329, Mallinckrodt Medical [U.K.] Ltd., Northampton, U.K.), and eight Amertec II 15-GBq (405-mCi) generators (product code MCC20, Amersham International p.l.c., Amersham, U.K.). Both types of generators are loaded with fission molybdenum 99. The generators were eluted 24 hours before being eluted to provide sodium pertechnetate Tc 99m for use in the preparation of technetium Tc 99m exametazime. Eluates from each manufacturer's generators were diluted immediately after elution to provide two solutions of sodium pertechnetate Tc 99m. To prepare one-hour-old solution, generator eluate was diluted with 0.9% sodium chloride injection to a concentration of radioactivity of 1.4 GBq (38 mCi) in 5.5 mL, injected into a sterile 10-mL nitrogen-filled vial (product code N80, Amersham), and used one hour later. To prepare six-hour-old sodium pertechnetate Tc 99m, the method was the same, but eluate was diluted to a concentration of 2.4 GBq (65 mCi) in 5.5 mL and used six hours later.

Technetium Tc 99m exametazime was prepared by injecting 5.0 mL of one of the solutions into an exametazime kit (Ceretec, Amersham) at the appropriate time. The concentrations of radioactivity in the sodium pertechnetate Tc 99m solutions were chosen to provide 1.1 GBq (30 mCi) of technetium Tc 99m in 5.0 mL at the time of kit reconstitution. A different lot of exametazime kits was used for each set of four experiments (i.e., one-hour-old and six-hour-old sodium pertechnetate Tc 99m from each manufacturer's generators). Each experiment was performed six times. Reconstituted kits were stored at room temperature.

At 2, 30, and 60 minutes after reconstitution of each kit, the radiochemical purity of the technetium Tc 99m exametazime was measured by using high-performance liquid chromatography with radiation detection. This technique for analyzing technetium Tc 99m exametazime has been shown to be satisfactory.[5] Analysis was performed by using gradient elution of a 150 × 4.6 mm column packed with a reversed-phase macroporous copolymer of styrene and divinylbenzene (PRP-1, Hamilton Co., Reno, NV). The column was fitted with a 25 × 3 mm guard column. The column was eluted with 20 mM phosphate buffer (pH 7.4) at a flow rate of 2 mL/min. Immediately after injection of a 20-μL sample of the radiopharmaceutical, tetrahydrofuran was introduced into the mobile phase in a linear gradient of 0–25% over six minutes. The final composition of buffer:tetrahydrofuran (75:25) was maintained for a further four minutes. Recovery from the column was measured as described previously[5] to exclude the possibility of the presence of species that might be adsorbed onto the column and remain undetected.

The chromatogram was obtained by acquiring the counts from the radiation detector in a multichannel analyzer (Accuspec, Canberra Nuclear Data, Schaumburg, IL). The chromatogram contained four peaks. A region of interest was placed around each peak, and the counts in each region were recorded and corrected for background. The counts in each peak were expressed as a percentage of the total counts in the chromatogram. The percentage in the peak corresponding to primary technetium Tc 99m exametazime complex was taken to be the radiochemical purity. Preparations of technetium Tc 99m exametazime with radiochemical purities of greater than 80% were considered acceptable.

Statistical analysis was performed with Student's *t* test. The a priori level of significance was <0.05.

Results

The chromatogram contained four peaks, which represented pertechnetate Tc 99m impurity, secondary technetium Tc 99m exametazime complex, an unidentified impurity, and primary technetium Tc 99m exametazime complex; these had retention times of 1.3, 3.2, 6.5, and 8.6 minutes, respectively. The mean ± S.D. recovery of radioactivity from the column of 99.1 ± 2.7% demonstrates that no major undetected species were present. Secondary technetium Tc 99m exametazime complex represented approximately 2.5% of the radioactivity at two minutes and approximately 8% at one hour. The unidentified impurity represented approximately 3.5% and did not change with time. These levels of secondary technetium Tc 99m exametazime complex and unidentified impurity did not vary with the generator used or the age of the sodium pertechnetate Tc 99m.

The radiochemical purities of technetium Tc 99m exametazime prepared under the various conditions are shown in Table 1. Each technique resulted in high radiochemical purity two minutes after preparation. However, at 30 and 60 minutes, the radiochemical purities of technetium Tc 99m exametazime prepared with six-hour-old sodium pertechnetate Tc 99m were significantly lower than the purities of technetium Tc 99m exametazime prepared with one-hour-old sodium pertechnetate Tc 99m. Similar results were found for each manufacturer's generators. No significant differences in radiochemical purity were found between corresponding results for technetium Tc 99m exametazime

prepared with sodium pertechnetate Tc 99m eluted from generators from different manufacturers.

The changes in the levels of pertechnetate Tc 99m impurity with time are also shown in Table 1. When one-hour-old sodium pertechnetate Tc 99m from either manufacturer's generators was used, the levels of pertechnetate Tc 99m impurity rose slowly and were not significantly different at 30 and 60 minutes than at 2 minutes. In contrast, when six-hour-old sodium pertechnetate Tc 99m from either manufacturer's generators was used, the levels rose much more quickly and were significantly higher at 30 and 60 minutes than at 2 minutes.

Discussion

Amersham International specifies that technetium Tc 99m exametazime with a radiochemical purity of at least 80% may be expected if all the recommendations for reconstitution of its exametazime kit are observed.[3] Ponto[4] challenged the recommendation that the technetium Tc 99m used for reconstitution be less than two hours old by obtaining satisfactory radiochemical purity with technetium Tc 99m up to five hours old. Ponto's result, however, was based solely on measurements made immediately after reconstitution. Of much greater importance is the radiochemical purity when the agent is administered. Because it is impossible to guarantee that technetium Tc 99m exametazime will always be administered immediately after reconstitution of the kit, it is best that the method of preparation result in satisfactory radiochemical purity at the expiration time quoted for the radiopharmaceutical. In the case of technetium Tc 99m exametazime, the expiration time is 30 minutes after reconstitution.

Table 1.
Levels of Selected Radiochemical Species in Technetium Tc 99m Exametazime after Use of Sodium Pertechnetate Tc 99m Eluted from Two Types of Generators

	Mean ± S.D. % of Radiochemical Species after Reconstitution ($n = 6$)		
Species[a]	2 min	30 min	60 min
Amersham Eluate, 1 Hour Old			
PTEC	92.7 ± 1.2	88.0 ± 2.1	83.2 ± 3.7
PI	1.2 ± 0.2	1.7 ± 0.8	2.9 ± 2.1
Amersham Eluate, 6 Hours Old			
PTEC	92.6 ± 2.0	80.9 ± 1.8[b]	70.8 ± 2.9[b]
PI	1.8 ± 1.2	10.0 ± 2.2[c]	17.8 ± 2.8[c]
Mallinckrodt Eluate, 1 Hour Old			
PTEC	92.8 ± 1.4	88.7 ± 0.7	84.7 ± 0.6
PI	1.5 ± 0.7	1.3 ± 0.3	2.1 ± 0.5
Mallinckrodt Eluate, 6 Hours Old			
PTEC	91.8 ± 1.9	81.9 ± 2.1[b]	72.1 ± 2.7[b]
PI	1.7 ± 0.4	8.4 ± 1.7[c]	15.6 ± 2.6[c]

[a] PTEC = Primary technetium Tc 99m exametazime complex (the indicator of radiochemical purity), PI = pertechnetate Tc 99m impurity.
[b] Significantly different ($p < 0.05$, Student's t test) from corresponding value for one-hour-old eluate from same manufacturer's generators.
[c] Significantly different from corresponding value at two minutes.

In my study, high radiochemical purity was present two minutes after reconstitution, regardless of the type of generator or the age of the sodium pertechnetate Tc 99m. These results agree with those of Ponto.[4] However, after 30 minutes the technetium Tc 99m exametazime prepared with one-hour-old sodium pertechnetate Tc 99m retained high radiochemical purity, whereas the technetium Tc 99m exametazime prepared with six-hour-old sodium pertechnetate Tc 99m did not. A similar discrepancy was seen at 60 minutes. Although at 30 minutes the radiochemical purity of the technetium Tc 99m exametazime prepared with six-hour-old sodium pertechnetate Tc 99m was greater than the manufacturer's 80% limit, it is too close to the limit for comfort, given the inevitable variability between batches of exametazime kits. These findings demonstrate that the age of the sodium pertechnetate Tc 99m used has a significant effect on the radiochemical purity of the technetium Tc 99m exametazime prepared.

It is conceivable that Ponto's findings were attributable to the technetium Tc 99m generators from which the sodium pertechnetate Tc 99m was obtained. Ponto used Mallinckrodt and Medi-Physics generators. To investigate this possibility, I compared technetium Tc 99m exametazime prepared with sodium pertechnetate Tc 99m from Amersham International generators with technetium Tc 99m exametazime prepared with sodium pertechnetate Tc 99m from Mallinckrodt generators; the results for the two generators agreed well at all times after reconstitution. Although Mallinckrodt generators were used in both studies, one was manufactured in Europe and the other was manufactured in the United States. Nevertheless, sodium pertechnetate Tc 99m from each of the total of four types of generators used in my study and Ponto's resulted in technetium Tc 99m exametazime of comparable radiochemical purity immediately after reconstitution. It appears that the model of technetium Tc 99m generator does not influence the radiochemical purity of technetium Tc 99m exametazime.

Three potential radiochemical impurities determine the radiochemical purity of technetium Tc 99m exametazime: pertechnetate Tc 99m, unbound reduced technetium Tc 99m, and secondary technetium Tc 99m exametazime complex. I previously showed that the increase in secondary technetium Tc 99m exametazime complex is not influenced by the age of the technetium Tc 99m.[5] Similar results were obtained in this study. Table 1 shows that when technetium Tc 99m exametazime is prepared from six-hour-old sodium pertechnetate Tc 99m, the level of pertechnetate Tc 99m impurity rises rapidly and that this species is the principal cause of the reduction in radiochemical purity.

Conclusion

The radiochemical purity of technetium Tc 99m exametazime was affected by the age of the sodium pertechnetate Tc 99m from which it was prepared but not by the generator from which the sodium pertechnetate Tc 99m was obtained.

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